Models of Self-Organization in Biological Development

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To my parents, in gratitude
and
in memory of Sven

Contents

Chapter 1

Introduction

To avoid the taint of theory in morphology is impossible, however much it may be wished.

BATESON, 1894 [22]

The development of the creatures around us has long captured the attention of human speculations and investigations, especially as it holds the potential for satisfying some of the natural curiosity about our own origins. Any process which is able somehow to transform a single fertilized egg into structurally and functionally complex multicellular organisms with the capacity for purposeful behaviour, must indeed be remarkable. Intensive research into biological development has yielded a mass of valuable insights, but has done little to dispel the fundamental sense of awe that inevitably accompanied the study of embryonic development. The mystery remains; for there is much in development that is as yet very imperfectly understood.

Any treatment of development which aims to be more than a mere space-time catalogue of observed processes (although providing even such a description is by no means a trivial and accomplished task!) must be able to account for the manner in which an apparently limited repertoire of cellular activities is used repeatedly in different contexts to generate the complex forms of multicellular organisms. The relationship between the hereditary 'blueprint' contained in the genome and the participating 'epigenetic' physical and chemical mechanisms which co-operate in the pattern-forming and morphogenetic behaviours of molecules and cells must be elucidated. In particular, true understanding of development depends on the clarification of the close coordination of developmental mechanisms, which display abundant evidence of being both highly intricate and having remarkable spatial and temporal coherence.

The Approach: Modelling of Self-Organization

> ... the quest of developmental biology is to understand the self-organisation process by which myriad identical proteins, transcribed directly from the genetic sequence code, interact with each other to ignite an upwards spiral of complexity amplification ... intuitive discussions however compelling the prose may be, are flimsy and should never be taken seriously unless rigorous mathematical solutions have actually been computed. (Odell, quoted in [364])

The modelling of developmental systems inevitably requires these to be idealized and simplified, but it consequently embodies the potential for isolating and clarifying those features most crucial for the pattern-forming processes, thereby enabling an advance in comprehension of the significant mechanisms. When considered in the light of an overarching conceptual framework, the relation between different theoretical approaches may be put into a global perspective.

In this thesis we thus wish to consider the concept of self-organization as an overall paradigm within which various theoretical approaches to the study of development may be described and evaluated. In the process, an attempt is made to give a fair and reasonably comprehensive overview of leading modelling approaches in developmental biology, with particular reference to self-organization. The work proceeds from a physical or mathematical perspective, but not unduly so — the major mathematical derivations and results are relegated to appendices — and attempts to fill a perceived gap in the extant review literature, in its breadth and attempted impartiality of scope.

Chapter Outline

A characteristic of the present account is its markedly interdisciplinary approach: it seeks to place self-organization models that have been proposed for biological pattern formation and morphogenesis both within the necessary experimentally-derived biological framework, and in the wider physical context of self-organization and the mathematical techniques that may be employed in its study. Hence the thesis begins with appropriate introductory chapters to provide the necessary background, before proceeding to a discussion of the models themselves. It should be noted that the work is structured so as to be read sequentially, from beginning to end; and that the chapters in the main text were designed to be understood essentially independently of the appendices, although frequent references to the latter are given.

In view of the vastness of the available information and literature on developmental biology, a working knowledge of embryological principles must be assumed. Consequently, rather than attempting a comprehensive introduction to experimental embryology, chapter 2 presents just a few biological preliminaries, to 'set the scene', outlining some of the major issues that we are dealing with, and sketching an indication of the current status of knowledge and research on development. The chapter is aimed at furnishing the necessary biological, experimental background, in the light of which the rest of the thesis should be read, and which should indeed underpin and motivate any theoretical discussions. We encounter the different hierarchical levels of description in this chapter, as well as some of the model systems whose experimental study has proved most fruitful, some of the concepts of experimental embryology, and a brief reference to some questions that will *not* be addressed in this work.

With chapter 3, we temporarily move away from developmental biology, and consider the wider physical and mathematical concepts related to the study of self-organization. Here we encounter physical and chemical examples of spontaneous structure formation, thermodynamic considerations, and different approaches to the description of complexity. Mathematical approaches to the dynamical study of self-organization are also introduced, with specific reference to reaction-diffusion equations, and we consider some possible chemical and biochemical realizations of self-organizing kinetics. The chapter may be read in conjunction with appendix A, which gives a somewhat more in-depth study of reaction-diffusion equations, their analysis and

properties, as an example of the approach to the analysis of self-organizing dynamical systems and mathematically-formulated models. Appendix B contains a more detailed discussion of the Belousov-Zhabotinskii reaction, which provides a vivid chemical paradigm for the concepts of symmetry-breaking and self-organization. Chapter 3 concludes with a brief discussion of a model biological system, the cellular slime mould, which displays rudimentary development and has thus proved amenable to detailed study and modelling.

The following two chapters form the core of the thesis, as they contain discussions of the detailed application of theoretical concepts and models, largely based on self-organization, to various developmental situations. We encounter a diversity of models which has arisen largely in the last quarter century, each of which attempts to account for some aspect of biological pattern formation and morphogenesis; an aim of the discussion is to assess the extent of the underlying unity of these models in terms of the self-organization paradigm. In chapter 4 chemical prepatterns and positional information are considered, without the overt involvement of cells in the patterning. In chapter 5, on the other hand, cellular interactions and activities are explicitly taken into account; this chapter should be read together with appendix C, which contains a brief introduction to the mathematical formulation and analysis of some of the models discussed.

The penultimate chapter, 6, considers two other approaches to the study of development; one of these has faded away, while the other is still apparently in the ascendant. The assumptions underlying catastrophe theory, the value of its applications to developmental biology and the reasons for its decline in popularity, are considered. Lastly, discrete approaches, including the recently fashionable cellular automata, are dealt with, and the possible roles of rule-based interactions, such as of the so-called L-systems, and of fractals and chaos are evaluated.

Chapter 7 then concludes the thesis with a brief assessment of the value of the self-organization concept to the study of biological development.

Relation to Existing Work

As there is already an extensive and diverse literature on developmental biology, which includes numerous reviews, it is helpful to point out the characteristic distinguishing features of the present work which justify the introduction of *yet another* overview of theoretical approaches to biological development.

It is apparent from the above outline of topics covered that this work attempts to steer a middle course. On the one hand, it is avowedly theoretical and mathematically grounded, but on the other it aims to take due cognisance of the full range of pertinent experimental insights that must precede and underly any theory. Thus the approach to the modelling avoids the extreme traps of, on the one hand, considering models merely for their intrinsic mathematical interest or beauty, and on the other, the abandonment of a mathematical formulation altogether in favour of less precise 'word arguments' or, worse, no attempt at seeking a theoretical understanding of observations at all.

The Biological Literature The vast majority of the works that comprise the developmental literature focusses exclusively on experimentation, and pays no heed to modelling at all — this is understandable, as it is the experimental discoveries which drive the progress of insights into

development, whereas the current state of modelling and theoretical understanding is still rather limited in terms of the phenomena it is able to consider.

There are, however, several general biological works which quote the results of theoretical analyses appropriate to and in the context of suitable experimental situations. For the attainment of a coherent and holistic understanding of development, such a presentation is in fact ideal, as models are placed in their correct context as *one* aspect of the explanation for a phenomenon, which cannot stand alone with no foundation in reality, but must crucially be evaluated in the light of experimental results and predictions. The books by Gilbert [115], Alberts *et al.* [1], Bard [16] and Slack [327] provide excellent examples of such a balanced perspective.

Probably the broadest and most comprehensive overview specifically of models in development, aimed predominantly towards clarifying the profusion of models for experimentalists, is the recent book by Held, *Models of Embryonic Periodicity* [159]. This author is concerned largely with providing a classification, or 'taxonomy', of models of patterning, and to this end reduces each model to its simplest assumptions, considering all in a non-partisan manner to permit a clear view of some of the merits and demerits of different approaches.

The biological literature is fundamental to any account of development, and ultimately must constitute the most important reference source for any embryological studies. The premise of the present work, however, is that as far as the full appreciation of the applicability and implications of models is concerned, it is not sufficient merely that theoretical approaches be related to the relevant experimental information. The complete understanding of models requires that they be studied in the light of their mathematical formulation and analysis, and in relation to analogous situations in chemistry and physics; only in a wider context can the full ramifications of theoretical concepts truly be understood.

The Physical Literature In complete contrast to the purely biological works stands a number of books that discuss self-organization models from a general physical perspective. There have been various approaches, most notably from Prigogine, Nicolis and coworkers [10, 274, 276, 277] and Haken [141], to the establishment of a general framework for self-organization, the spontaneous creation of order and structure in an initially structureless domain. The generation of biological pattern and form is then usually taken as an example of the application of these general principles.

Such works are important for a holistic understanding encompassing a broad range of phenomena in the sciences, but they can inevitably not do full justice to embryological phenomena, nor do they attempt to do so. The placing of developmental mechanisms in a broader context is vital, as we have argued above; but biological complexity is uniquely distinguished from that of physics and chemistry by the historical and stochastic nature of the processes of evolution and natural selection which have created the organisms living today — unlike in the physical sciences, there are no fundamental laws in biology, and general principles are insufficient: one must constantly respect the experimentally-observed idiosyncracies of individual organisms and mechanisms.

Theoretical and Mathematical Developmental Biology The works most pertinent to our present study are those of the practitioners of the modelling themselves, that is, the theoretically inclined biologists and biologically inclined applied mathematicians, physicists and chemists who

formulate the theories and models about developmental mechanisms. In this case, a number of excellent reviews exists, but it appears that none of these provides the full combination of interrelated factors which the present work treats, namely: a *broad and comprehensive overview of models* in development, in the light of their *mathematical formulation and analysis*; an account of the *experimental embryological insights* underlying these models; and the consideration of the full *physical and conceptual context* within which the self-organization models may be placed.

We may point out some of the most significant books and reviews in the mathematical biology literature related to development: Murray's book, *Mathematical Biology* [251], provides an excellent introduction to mathematical techniques useful in the analysis of models, and also places emphasis on the constant interaction of theoretical insights and experimental data. Meinhardt, in *Models of Biological Pattern Formation* [229], has similarly strongly stressed the constant interplay between theory and experiment, neither being able to stand alone without the other. It is a feature of both these works that they focus strongly on those developmental models which the authors themselves formulated, or to which they contributed. A similar situation prevails in a number of excellent review articles, by Gierer [112], Harrison [150], Meinhardt [233], Murray [252], and Nagorcka [264], in all of which the authors have chosen to concentrate on the work with which they are most familiar, rather than to attempt a broad survey of *all* the major modelling approaches. A more general introduction is given in the last chapter of the book by Edelstein-Keshet [87]; this clear and valuable overview of the available modelling strategies is however directed at the level of a senior undergraduate textbook, and thus does not go into much detail.

There thus appears to be a gap in the review literature, which calls for a work that provides a balanced treatment of all the major modelling approaches to developmental pattern formation and morphogenesis on a sound mathematical footing, in the broader context of self-organization and the experimental literature. This thesis attempts to go some way towards filling that gap. With an adequate motivation for the present work thus established, we may now embark on our study of *Models of Self-Organization in Biological Development.*

Chapter 2

Biological Preliminaries

The fundamental problem of developmental biology may be stated as: "how does the information encoded in the one-dimensional genome specify a three-dimensional organism?"; it is the question of how the exquisitely choreographed sequence of molecular and cellular mechanisms that acts to produce biological pattern and structure is controlled. We have already in the introduction given a brief description of the overall problem of the understanding of pattern formation and morphogenesis; of course, any individual research effort will have far more modest ambitions, and will be focussed on a particular experimental system, a developmental phenomenon observed in a specific organism that is easily accessible to experimentation. This chapter is included to provide a *biological context* for the theoretical considerations that will constitute the bulk of this thesis.

The vast scope of extant knowledge on developmental biology can never be summarized satisfactorily in a few dozen pages, so this chapter does not pretend to be at all comprehensive or definitive. It is rather intended to provide a brief survey of the salient features for our later discussions, a broad sweep of the diverse and fascinating field of animal development that will form the subject of our theoretical studies. Although an attempt is made to cover all pertinent biological concepts, thirty pages can never suffice to familiarize anyone with the wide range of experimental concepts and results that must underly any theories of development, so that the reader is already assumed to have some working knowledge of the major concepts of embryology, as well as of cellular and molecular biology and genetics. There is, of course, a large choice of excellent recent books which can provide the necessary background, a few of which are mentioned below:

For a very quick introductory glimpse at some of the relevant themes, there is the popular article by Wolpert [379], which is however considerably less detailed (and more narrowly focussed) than the present chapter. Wolpert's book, *The Triumph of the Embryo* [378], is aimed at the intelligent layperson, and is a good introduction to embryological concepts and theories, covering a wide range of important issues, with virtually no biological background assumed. The relevant chapter in the textbook by Campbell, *Biology* [48], also provides a good introduction, outlining the main features of early development, and giving some indication of mechanisms involved in development.

Two excellent general texts, aimed at senior undergraduate biology students, are the chapter on 'Cellular Mechanisms of Development' in the book by Alberts *et al.*, *Molecular Biology of*

the Cell [1] (read in conjunction with appropriate sections of the rest of the book), and the book *Developmental Biology* by Gilbert, [115], both of which give detailed and comprehensive outlines of all the major issues and events in development, including theoretical approaches and with an introduction to the primary literature.

Somewhat more specialized, but nevertheless highly readable and useful, are the books by Slack, *From Egg to Embryo* [327], and Bard, *Morphogenesis* [16]. The former presents a lucid summary of the concepts of experimental embryology, together with a biologist's perspective of the value of a dynamic, mathematical framework for development; the bulk of the work considers regional specification in early development, with specific reference to the major experimental organisms that have been studied. The latter book confines itself to the cellular and molecular processes involved in the development of anatomical structures, thus operating at a supragenetic level, and gives a good outline of the palette of mechanisms available to the cells in the generation of form.

There are of course many other appropriate books, both general textbooks and more specialized monographs — and of course the primary literature — but the above-mentioned works should be sufficient to provide a thorough introduction to the pertinent concepts of developmental biology. What follows aims not to replace any of the above descriptions, but merely to pick out and mention some key aspects of development to set the scene for our subsequent discussion, and possibly to serve as an introduction to further reading in any of the above books. As the biological background presented in this chapter is largely 'standard knowledge', few references will be given, and the reader is referred to the above-mentioned texts, from which much of this discussion is derived.

Outline of Early Development To give a flavour of what follows, it is appropriate at this stage to give a very brief overview of the earliest stages of development; terms and concepts mentioned cursorily here will be treated more fully later:

After the union of egg and sperm in *fertilization*, *cleavage*, a process of repeated cell divisions, divides up the *zygote*, or fertilized egg, to give a population of smaller cells arranged as a hollow spherical epithelial sheet, the *blastula*. This ball of cells is subsequently moulded to give the complex geometrical structures of the organism, with the first and most dramatic stage being *gastrulation*. Here, by a complicated invagination, a large area of cells on the outside of the embryo is brought to lie inside it, and the three *primary germ layers*, the endoderm, mesoderm and ectoderm, are distinguished as the inner, middle and outer cell layers respectively. These three layers already constitute the basic body plan, and each leads to the formation of specific tissues and organs in the final organism; roughly, the endoderm gives rise to the gut and associated organs, the mesoderm forms connective tissue, muscles, and the vascular and urogenital systems, and the ectoderm leads to the epidermis and the nervous system. Following gastrulation, the stage is set for the complex interactions of cell differentiation, pattern formation and morphogenesis that constitute *organogenesis*, the shaping of tissue and organ rudiments, which gradually generates the structures that eventually constitute an independently living, functional *organism*.

Cell Differentiation, Pattern Formation and Morphogenesis

The development of multicellular organisms may broadly be said to comprise three conceptually distinct aspects: cell differentiation, pattern formation and morphogenesis [10, 375, 377].

The first of these aspects, **cell differentiation**, is the one of least interest to us in this thesis, as it is generally not incorporated directly in any of the modelling approaches, being considered rather to proceed independently in consequence of the pattern-forming and morphogenetic processes that *will* be our concern. An adult multicellular organism contains a range of cell types, which may be distinguished by their form, composition and function. Cell differentiation is, briefly, the process by which cells somehow *change their character*, so that the full diversity of adult cell types can arise from a single fertilized cell. The genetic basis for cell differentiation is considered in some more depth below, in section 2.1.1.

The *spatial control of cell differentiation* is termed **pattern formation**. Particular sequences of cell differentiation occur in different organs — for example, the analogous distinction between muscle, cartilage, blood, skin and nerve cells is made in the vertebrate arm and leg — and the patterning problem is in how the correct and reproducible spatial (and temporal) proportions, relationships and localization of the various cell types are controlled and maintained.

The third fundamental phenomenon is that of **morphogenesis**, the *generation of form and structure* in development. This occurs through coordinated use of a repertoire of cellular activities, including the motion of individual cells and the deformations of cell sheets, which will be outlined in section 2.1.3; the action of the variety of physico-chemical mechanisms available to the embryo leads to the moulding of tissues to produce the organs which make up the organism.

The Relation between Pattern Formation and Morphogenesis Pattern formation and morphogenesis, although conceptually distinct processes, frequently occur in parallel and may not always be distinguished in practice; it is often the case that pattern formation *manifests itself* through differences between regions established by mechanical or other physico-chemical processes involving the coordinated behaviour of cells. In other situations, however, the process of pattern formation *establishes* the regional differences that are then revealed or amplified by the differential response of the form-producing mechanisms to the preexisting pattern. For example, if structure is produced as a consequence of differential adhesion or the deformation of a cell sheet due to localized forces, then the problem of pattern formation is in establishing how the local differences arose in first place.

There are two scenarios: morphogenesis may occur simultaneously with or subsequent to pattern formation; the distinction between these possibilities is not always made. This leads to frequent misuse of the term 'morphogenesis' or the identification of these two concepts (for example the usage "Morphogenesis is the development of pattern and form in living systems" [252, p.119]), and it is often convenient to consider pattern formation and morphogenesis together. Nevertheless, the distinction is an important one, and is made clearly in this thesis, with chapter 4 considering pattern formation in isolation from consequent morphogenetic structural changes, and chapter 5 dealing with the direct involvement of cells in their own patterning, so that pattern formation and morphogenesis occur as an interactive, simultaneous process.

It is important to discriminate clearly between pattern formation and morphogenesis at this early stage in our deliberations, and obtain a proper perspective on these concepts and their

relation to cell differentiation — which only recapitulates and manifests existing patterns or structures — in order to be totally clear what the issues involved in developmental pattern formation and morphogenesis are. Throughout our study of pattern formation and morphogenesis, we will not be directly concerned with the mechanisms or details of the resultant cellular differentiation, but assume it to proceed determinately from the structures and patterns — in local concentrations of chemicals, cell densities, traction or other forces, cell sheet tensions or curvatures, cell adhesivities, or any other relevant patterned cellular or molecular properties — in some here unspecified way. It suffices here to inquire after the *origin* of these patterns, in particular after possible self-organizing mechanisms or processes that might give rise to such patterns and structures, and to rely on the experimental information that indicates that a multitude of factors, such as many of those indicated above, may directly influence the differentiation state of a cell. (For a recent biologically-oriented review on pattern and diversity generation in animal development, see [139].)

Strategies for Embryological Research

It is helpful to approach a discussion of some of the features that have emerged from the extensive study of development through some of the *techniques* that have been applied to such study, as they to some extent define and circumscribe the diversity of possible advances made. In broad outline, there is the distinction between natural history and science, that is between mere descriptive and active investigative approaches to embryology; but within the realm of experimentation, a succession of techniques has gained ascendancy as experimental embryology has become more refined. In this brief outline of research strategies, we largely follow Bard [16].

1. **Descriptive embryology** might appear at first sight to be little more than the 'stamp collecting' cataloguing of events that is denigrated by recent generations of scientists as dull and uninspired, and not within the domain of proper scientific investigation. It is sobering to consider that this classical descriptive approach is the basis on which all other studies depend, for before one can ask *how* the embryo accomplishes a certain task, one has to be sure one knows exactly *what* is happening.

   A wide range of techniques is at the disposal of the investigator, including observation under a dissection microscope, time-lapse cinemicroscopy, scanning and transmission electron microscopy, histochemistry and immunohistochemistry, with Nomarski optics and confocal microscopy being available for the study of three-dimensional organization. Many of these techniques have only come into their own fairly recently, and enable a thorough and detailed investigation of the spatial and temporal sequence of processes by which tissues and organs form, which is an essential initial condition for the next step, the elucidation of the mechanisms by which such organization takes place.

2. Following description of overt *events*, we need to seek the *mechanisms* underlying these events, which leads us to **classical experimental embryology**. According to the standard and most obvious approach, one guesses ('hypothesizes') a mechanism that might be underlying the observed activities, and then interferes with the embryo in such a way as to test the hypothesis, that is, so that different responses would be expected were the guess correct or wrong.

Experimental embryology, in the traditional approach, requires mainly simple instruments, a dissecting microscope and a competent pair of hands. The manipulations can be performed *in vivo*, that is in the developing organism, by carrying out surgical interventions such as excision or transplantation of parts, to see how the embryo copes with changes in its cellular organization. The main alternative is cell and tissue culture, studying the behaviour of cells on, especially, a petri dish, where one attempts to isolate the system of interest and get it to do *in vitro* what it does in normal development. The simpler system thus obtained may then be more readily manipulated to try to identify the mechanisms at work. The reductionist strategy, of searching for the cellular properties underlying tissue formation in the hope of understanding how the observed structures arise, has been particularly popular in the last few decades.

3. An alternative approach has been on a *genetic level*, to attempt to identify specific simple **mutations** that lead to well-defined structural abnormalities by altering cellular or molecular properties. The hope then is that by investigating the mutant, one can dissect the mechanism at work. Such analyses have been useful for instance in identifying interdependent developmental regions ('fields'), through the so-called homeotic mutations that cause gross deformations such as the replacement of one appendage by another (one of the most famous examples is possibly the *Antennapedia* mutation in the fruit fly *Drosophila*, in which the mutant fly has second legs in place of antennae). The recognition of *which* gene is involved in a particular developmental process, does not however necessarily bring us any closer to an understanding of *how* the process occurs. The exception to this is in the case where the effect of a mutation can be traced to a well-defined alteration in a local cellular property, such as adhesivity or shape, in which case that property may be definitively linked with the abnormalities resulting from the mutation, and hence with the corresponding normal structure.

4. In the last decade or two, the entire field of developmental biology has seen an explosion of activity, as the techniques of **molecular biology** and **DNA manipulation** have been brought to bear on developmental questions. Thus a wide range of biochemical and immunological techniques, especially the use of monoclonal and polyclonal antibodies, has been used to investigate the spatio-temporal localization of morphogenetically significant molecules, changes in the expression of which lead to changes in cell behaviour and hence altered tissue organization. Hence as we shall see below, structural, macroscopic phenomenological changes may now frequently be correlated with molecular distributions. In the event that the genes coding for these molecules are known, we may trace the mechanisms to the genetic level, and seek the factors controlling the relevant gene expression.

5. It is on the **genetic level** that the major advances have been made in recent years, as the methods of *genetic engineering* enable the active creation of mutations to assay the roles of specific genes, especially in early development. The piece of DNA responsible for a particular phenomenon can be identified, removed, cloned and analysed; with the availability of a clone, *in situ* hybridization can be used to follow the gene expression pattern and correlate it with developmental phenomena. Direct gene manipulation enables the effect of specific mutations to be studied; alternatively, recovery can be investigated by reintroducing healthy genes into mutant animals. There has been considerable excitement about the results and insights gained by this explosion in genetic analysis, which began

and has flourished in the study of *Drosophila*, for which the sequence of events and controls leading to early regional specification and spatial ordering has been nearly fully elucidated (see the brief description below, in section 2.2.3), and is spreading to other organisms.

Developmental genetics, appropriately coupled with molecular biology and classical embryology, has proved to be an extremely effective and powerful tool for studying the control of the sequence of early patterning events in the embryo. On the other hand, no mechanism involved in morphogenesis, the generation of form and structure, has *directly* been discovered or clarified in this way — genetic analyses are still too far removed from the observed macroscopic events, which are typically the consequence of an intricate combination of factors. Ultimately, we seek to demonstrate the relationship between the genotype and phenotype by elucidating the mechanisms by which genes control development. The desired synthesis will require an understanding at all levels, however, from the genes up, so that all the classical and modern techniques will be involved in attaining the final understanding.

6. The final 'technique' used to seek understanding, is that utilized and described in this work, namely the use of **theory and models**, including computer simulations. The mechanisms involved in morphogenesis are complex, and the formulation of models enables significant features to be extracted and their dynamical behaviour investigated, with the need to develop a quantitive formulation of the mechanism imposing a requirement of rigour and clarity of thought. Analytical and numerical studies of the models then enable one to demonstrate the potential morphogenetic properties of a given seemingly plausible mechanism, to see whether it does what one would expect, as the strictures of mathematical formalization enable a far more rigorous study than verbal or heuristic arguments. The successful simulation of experiments using a model provides suggestive supporting evidence for the validity of the model, which becomes more convincing if novel experimental predictions can be made and confirmed. A theoretical analysis also enables underlying *analogies* and isomorphisms between apparently different mechanisms to be recognized, as will be demonstrated throughout this thesis.

   Conceptual approaches and modelling can never stand in isolation from experimental analyses, but must complement these and must spring from a proper appreciation of the experimental data — models are little use if they appear compatible with and can be adapted to fit *any* data, and do not lead to testable and falsifiable predictions, since they are then in danger of constituting abstract theorizing divorced from biological reality. On the other hand, we may not neglect a theoretical appreciation of phenomena and mechanisms, as a complete, coherent understanding of development can only arise from the unification of the mass of 'amorphous' experimental results into a cohesive conceptual framework that binds the observed phenomena into a unified, intelligible whole.

2.1 Hierarchies of Description

The existence of different, hierarchical levels of description of developmental processes and mechanisms has become apparent from the above discussion of the range of extant investigative techniques. It is a characteristic feature of biological systems that such *hierarchies* exist, and indeed,

the complexity of biological structure and function derives from the interrelation of such distinct levels of organization. The classification of the hierarchical sequence varies somewhat, but a natural sequence is: atom – molecule – macromolecule – subcellular organelle – cell – tissue – organ – whole living organism – population of organisms – community – ecosystem (adapted from [303, p.8]). The entities at each level are composed of the coordinated interaction of units at the next lower level down the hierarchy.

A significant feature of the hierarchical organization is its *irreducibility of description* — each level must be considered as a distinct entity, and cannot be reduced fully to a discussion of its building blocks. Although reductionism can produce great insight into the properties of, say, a cell in terms of its constituent molecules, what matters ultimately is also their *organization* and *cooperative structure*, so that a holistic view is required — 'the whole is greater than the sum of its parts'. A more detailed discussion of this point in the context of complexity, on a more physical basis, will be given in the next chapter, section 3.1.5.

In our present discussion of development, we shall consider a brief hierarchy of genes – molecules – cells, slightly different from the above, to take account of the manner in which DNA expression underlies developmental processes.

2.1.1 Genes

Genes constitute spatially localized portions of a DNA strand, characterized by a specific sequence of A, T, G or C (adenine, thymine, guanine or cytosine) nucleotides arranged in complementary pairs. Subject to the binding of suitable combinations of activator and repressor protein molecules to an appropriate promoter region of the DNA, genes are *transcribed* within the nucleus to form messenger RNA (mRNA), which is 'processed' before it exits the nucleus to be *translated* into proteins by the ribosomes in the cytoplasm. The, somewhat redundant, genetic code ensures that three adjacent base pairs form a codon corresponding to a unique amino acid of the twenty that constitute the natural building blocks of polypeptides and proteins. The most fundamental biological level of description containing 'information' thus ultimately lies at the level of the genome.

The mode of action of genes is through their protein molecular products: These proteins and other macromolecular species — carbohydrates and lipids, 'manufactured' through protein-catalyzed enzymatic reactions — constitute the basis for cellular structure and function, so that the influence of genes is in 'determining' the protein distribution. Conversely, on the other hand, gene expression depends on appropriate distributions of promoter and repressor proteins (as well as, of course, the crucial involvement of enzymes such as RNA polymerase), so that there is a constant *feedback* between the genetic and protein levels. Ultimately, however, the available genes determine the proteins that may be synthesized, and thus dictate the range of potential structures and behaviours possible in an organism. For brief discussions of the extent to which genes may be said to 'control' development, see sections 2.3.3 and 6.2.3.

Cell Differentiation The initial fertilized egg is *totipotent*, that is, it is capable of generating all cell types in a multicellular organism. After successive mitotic cell divisions, however, daughter cells are increasingly constrained to a particular developmental pathway, through determination and **differentiation**. The distinction between different cell types, such as between

a cartilage and muscle cell, is visible through differences in appearance, such as shape or internal organization of the cell, but several processes indicate a distinction between cell types before differentiation is overtly manifested, including prior formation of different protein products, observable through the use of antibodies for specific proteins. Rather than restricting the concept of differentiation to *observable* differences between cells, this fundamental process is thus today defined as the "synthesis by a cell of species of protein different to those made at an earlier developmental stage, or different to those made by surrounding cells at the same stage" [327, p.32].

The process of differentiation does not involve modification of the genome — with very few exceptions (for example immunoglobulin cells in the human immune system, and haploid germ cells possessing only one copy of each chromosome, rather than two copies) the DNA sequence in every cell in an organism is the same — so that the distinction between cells is in *which* genes are expressed. The basis for the regional differences and structures in the embryo is thus *differential gene expression*, occurring in a spatially and temporally ordered manner.

It is not possible to go here into a detailed discussion of the molecular foundations of differentiation, including binding of promoter and repressor proteins, chromosomal packing, DNA methylation, cytoplasmic composition and organization (see for example [23, 27], the former for a discussion of 'smart' regulatory genes, the latter for the influence of the cytoskeleton in gene exposure and genetic regulation). For the purpose of our discussion, it suffices to recognize that differentiation is clearly fundamental to the generation of functionally distinct, and interactive, structures in higher organisms.

With some exceptions (for example the ability of cockroaches and some amphibians to regenerate limbs after amputation — see section 4.2.2) differentiation is a terminal, irreversible process, but it does not occur in a single-step, on-off manner. Rather, the body plan and structure, together with the cell types that constitute the final organs, are formed as a result of a *hierarchy of developmental decisions*. Regarding pattern formation, first the rough plan is laid down, and the forms are then successively moulded to ever finer detail. Similarly, the familiar histological cell types observed in the adult organism, such as skin and neural cells, are formed as a result of a series of states of commitment; each of these states arises from a subdivision of an earlier and less restricted commitment, and is able to make a choice between a restricted set of alternatives to form, in general, new states of commitment whose *potency* is further restricted. For example, for cells to be competent to form the lens of an eye, they must have already decided that they are ectoderm (rather than mesoderm or endoderm) and then epidermis (instead of neural plate or neural crest cells). The successive determination of cells arises from the signals they receive, together with their internal developmental programme which is, in turn, dictated by their previous developmental history. There is the constant feedback interaction between the genetic level, which embodies the possible cell types and developmental potentialities, and the epigenetically specified direction of the gene expression patterns.

This has been a very quick introduction to the vast field of developmental genetics; for more information at an introductory level, the books by Campbell [48, ch.15,16,18] and Alberts *et al.* [1, ch.9,10] (slightly more advanced) may be recommended, whereas a compact summary of "the organisation, packaging, transcription and regulation of genes in animal systems" is given by de Pomerai [77, ch.1].

2.1.2 Molecules

We have already seen that gene expression manifests itself in the formation of proteins, which in turn regulate the expression of genes, so that the genetic and molecular levels are intimately interlinked. As the composition of the cell is based on macromolecules, molecular properties and behaviour indeed necessarily underlie all of development. The molecular and subcellular level of description extends well beyond this, however; there are three specific classes of molecules, geographically distinct, which directly influence cell behaviour and thus underpin changes in tissue organization. These molecular classes are the extracellular matrix, the components of the cell membrane and those comprising the intracellular cytoskeleton. Together, they form the structural molecules of the cells and their environment, which guide, constrain, facilitate and generate cell behaviour. A useful discussion of these morphogenetically significant molecules is given by Bard [16, ch.4]; also see Alberts *et al.* [1, ch.11,14].

Extracellular Matrix

The extracellular matrix, or ECM, may be defined as that material which is within the organism, but external to the cell membranes; although a rigid distinction cannot be made in view of the strong adhesions between the ECM and cellular membranes. The ECM provides a rigid or elastic matrix that maintains the integrity and strength of organs and provides the main building blocks of tissues such as bone, cartilage and tendon, also constituting the basal lamina that binds to sheets of epithelial cells. The ECM is composed of several different classes of molecules: these include collagens, the most common structural protein in the body; hydrated macromolecules such as hyaluronic acid, a linear molecule consisting of repeated disaccharide units, and sulphated proteoglycans; and substrate-adhesion molecules such as the glycoproteins fibronectin and chondronectin, to which cell make the adhesions that allow them to spread or move.

The ECM has a range of vital morphogenetic properties:

- The extracellular environment has the ability to control cell differentiation in certain contexts.
- The ECM may be expected to play a role in *creating space*, for instance through the hydration of hyaluronic acid or proteoglycans, through which cells can move.
- Almost by definition, the ECM provides the environment within which *cell movement* takes place, also forming a substratum to which cells adhere and which can guide cell motion.
- The most obvious role of the ECM is perhaps in *stabilizing intermediate structures* and thus allowing them to form complete tissues, by providing a stable substratum for their adhesion; in the absence of such stabilization, some tissues would fall apart.
- By directly participating in the *formation of tissues* such as cartilage condensations, the ECM can mediate or even trigger certain morphogenetic processes; we shall see evidence and models for such a process later (section 5.1.1).

A fair amount is known about ECM constitution and how it facilitates morphogenesis, but the manner in which it is laid down is still little understood; clearly, its structure and distribution dictates its influence on morphogenesis.

Cell Membrane Molecules

The cell membrane is the interface between the inside and the outside of the cell, thus providing the site of communication between cells, and with the ECM. As a consequence, it has several morphogenetically significant functions. The major constituents of the membrane as such are phospholipids, but it is specific classes of membrane glycoproteins, namely the cell adhesion molecules (CAMs) and the membrane receptors for the substrate adhesion molecules (SAMs) of the ECM, which are morphogenetically active. Adhesion follows from specific binding by a homophilic interaction (a N-CAM molecule on one cell adheres to a second N-CAM on an opposed cell, for example), although heterophilic interactions, between different molecules, also occur. The patterns of expression of cell adhesion molecules have been shown to be well correlated with a variety of significant developmental events moulding form, indicating their importance in morphogenesis (see [82, 84]).

The membrane plays a number of roles necessary for the successful operation of certain morphogenetic events:

- The environment of cells, even during morphogenesis, is unusually stable; the explanation for the *maintenance of tissue integrity* is to be found in strong adhesions between cells themselves and to the basal lamina (for epithelial cells — see below) and by adhesions of mesenchymal cells to the ECM.

- Membranes are passively involved in morphogenesis as they *permit communication*, by diffusion of small molecules through the cell membrane, facilitated diffusion and active transport mediated by membrane proteins, and by passage of molecular species through tight junctions between adjacent cells.

- Cell adhesion molecules *facilitate movement*, which depends on the breaking of attachments with the ECM and the reformation of adhesions elsewhere; specifically, cells will move from positions where the adhesivity is low to where it is high, a phenomenon known as *haptotaxis*, which may be accounted for by the minimization of free energy when the adhesive binding energy is a maximum.

- Mixtures of disaggregated cells, for example of two species of sponge, or different amphibian tissues, will to a reasonable extent sort themselves into their original tissue organization (see sections 5.1.3, 5.3.2). The molecular basis of *sorting out*, which in fact appears to be rare in normal development, is thought to lie in differential adhesion between tissue-specific and species-specific adhesion molecules.

Intracellular Molecules

Cells are the 'building blocks' from which organs and tissues form during morphogenesis, but rather than there being a 'builder', they actively participate in the process themselves. It has

become clear in recent years that the behaviour of cells (which we shall consider in outline below) such as movement and bending of sheets, must be understood in terms of the *internal, structural changes* occurring within the cells (see section 5.2). The emphasis is not so much on the intracellular organelles, such as the mitochondria and ribosomes, which perform standard 'housekeeping' activities, but on the *cytoskeleton*, which dictates intracellular organization and structure.

This cytoskeleton is an integrated network of fibres in the cytoplasm, with three main components: *Microtubules* are polarized, helically organized hollow polymers of α-tubulin and β-tubulin globular proteins; *microfilaments* are thinner, and are solid rods of β- and γ-actin proteins polymerized into a double helix; and *intermediate filaments* have, as their name suggests, intermediate size, and a variety of protein subunits and functions. These cytoskeletal components are all interlinked, though having specific functions. The importance of the intracellular hydrostatic pressure of the cytoplasm, mediated by the intake of water by the cell or by the equilibrium between solation and gelation of the cytoplasm, may also not be overlooked.

The cytoskeleton plays diverse roles in the morphogenetic deformation and movement of cells:

- *Intracellular activities*, especially mitotic cell division (where microtubules are responsible for chromosome movement and microfilament contraction for cytokinesis, the division of the cytoplasm) are vital for morphogenesis to be possible, although they are not necessarily related to development. Of morphogenetic interest also are the changes such as cytoplasmic movement taking place in the egg membrane and cortex immediately after fertilization, in which the cytoskeleton presumably plays a guiding role.

- Events taking place inside the cell may lead to *cell shape changes* which can have large-scale effects on the tissues; for example, microfilament contraction at one end can lead to buckling of cell sheets. Such effects will be discussed in more detail in section 5.2.

- The coordinated contraction of microfilaments is also responsible for *cell movement*, which depends also on the contraction of an intracellular actin gel, presumably so that water is extruded at the anterior end to generate forward movement of the cell.

- The final function of the cytoskeleton mentioned here also appears in the roles of the ECM and CAMs, namely *morphological stabilization of structures* once they have formed; the intracellular contribution is mainly to maintain cell shape and hence provide tissue strength.

The molecular level is thus a highly significant one, especially as it should be noted that we have considered here only the most important and best-understood classes of molecules, and we shall later postulate on theoretical grounds other molecules, denoted 'morphogens', whose distribution is assumed to play a directive role in patterning. We should not overestimate the value of the molecular approach, however, even though this would be naïvely justified by the fact that cells are, ultimately, built of molecules, for we have already noted the existence of different hierarchical levels, each with its own irreducible features. Furthermore, the molecular basis of much cellular behaviour is at present poorly understood and can only be considered phenomenologically, while the stochastic aspects of other cellular processes cannot be predicted from a molecular level; and finally, we shall see abundant examples of morphogenetic events

depending on macroscopic structures or extended forces, for which molecular analysis provides little insight. Nevertheless, the study of molecular aspects of morphogenesis over recent years has proved extremely fruitful.

2.1.3 Cells

The consideration of more fundamental structures, the molecules and genes underlying development, is necessary and important, but ultimately the **cell** is the basic unit of life, with a complex organization and metabolism, and capable of homeostasis and self-reproduction. In the developmental context, it is cells and cellular activities that form the structures of the embryo. Genes act at the molecular level, but the significance of the genetic and molecular descriptions, which must be a goal of developmental research, is ultimately to *account for cellular properties and activities*, for it is these that cooperate to generate pattern and form. The same, fairly limited set of cell activities — cell multiplication, cell growth, cell movement, cell differentiation, changes in cellular shape and properties, intercellular signalling, cell death — is used repeatedly, but organized differently in space and time to produce the distinctions between different parts of an organism.

When considering the morphogenetic properties of cells, it is convenient to distinguish between the two main types of embryonic cells, those of the **mesenchyme** and those that comprise **epithelial sheets**. Note that although, as we shall see, there is a clear distinction between these types and their properties, they are not immutable, and conversions from mesenchyme to epithelium or *vice versa* are known during development.

Mesenchymal Cells

Mesenchymal cells tend to be identified as those embryonic cells that are found as *individuals* or in groups rather than in sheets, and that have not differentiated into a specialized cell type. Of the three primary cell layers that follow gastrulation, mesenchymal cells derive from the mesoderm, as well as from the migratory neural crest cells of the ectoderm. Their final differentiation state may include muscle and other specialized cells, but mesenchymal cells are usually considered to be those cells that become supporting matrix for muscle, epithelial sheets or neural tissue, or form the cellular component of connective tissue. Among other types, this range includes chondrocytes, the precursors of cartilage and bone, and fibroblasts, those motile cells which lay down the ECM (the terms 'fibroblast' and 'mesenchymal cell' are sometimes used interchangeably).

Elementary morphogenetic properties of mesenchymal cells include their growth and death, both occurring at specific stages in development and acting especially in the sculpting of fine detail of tissue organization. Mesenchymal, or fibroblast, cells also play an important role in laying down the ECM with which they interact. But the most obvious morphogenetic ability of mesenchymal cells is however their ability to *move*, and their morphology is appropriate to this, with a characteristic polarity and protuberances, or *filopodia*, at the leading edge of motion.

The motion has a strong stochastic component, changing direction constantly and randomly, so that the *constraints* that direct the motion are of particular interest. As we have seen, for example, the ECM provides contact guidance, and an adhesive gradient will direct movement,

as a more adhesive substratum enables the cells to use more adhesive sites and hence minimize free energy. Cells are furthermore inhibited by contact with other cells, leading to changes in direction; parallel associative movement of mesenchymal cells may result.

The other major morphogenetic feature of mesenchymal cells is the formation of condensations, or aggregates, as the precursors to bone and skin organ rudiments, for example; and the tractional forces that have been shown to be exerted by such cells may be able to account for this. The morphogenetic effects of all these mechanisms will be considered in much more detail in section 5.1, in the light of modelling approaches to mesenchymal morphogenesis, so that they are merely skimmed over here.

Epithelial Cells

In contrast to the individualized mesenchymal cells, epithelial cells are those that tend to be *associated in sheets*, which are usually single-layered and *polarized* (that is, their two sides are distinct). They are found in a diversity of forms, that include bounding membranes of tissues, and a wide variety of tubes and vesicles. It is these sheets that largely define the early embryo, with the mesenchyme essentially only filling the spaces between them, and the major problem of morphogenesis may be to explain how these sheets come to be bent, folded and otherwise deformed to create such a diverse set of structures. As for the mesenchymal cells, we shall consider models analysing and accounting for some of the repertoire of epithelial cells in chapter 5, so that we merely outline some of these features briefly here.

The changes in shape of epithelial cells derive from intracellular deformations of the cytoskeleton, as discussed above. These changes include *palisading*, that is the elongation of cells from flattened 'squamous' epithelium to a more columnar shape; as in the formation of dermal placodes, the initial stage in the generation of feathers, hairs and other skin organs, and the various sense organs. The mechanism underlying this is not fully understood, as microtubule extension appears not to be involved. Asymmetric deformations of epithelial cells on the two sides of the sheet can lead to *folding*, *buckling*, and *invagination and evagination*; a likely mechanism for folding is localized microfilament contraction, whereas buckling can arise from distortions due to excessive mechanical stresses. Epithelial tubes are very common in the embryo, and can form in at least three distinct ways: by the fusion of epithelial folds, by the extension through growth or elongation of an invagination or evagination, or by the hollowing out of a solid cellular array in which the cells have become polarized.

A remarkable and significant feature of morphogenesis is epithelial movement, which is very common and important in the early developmental stages, although occurring rarely after neurulation (the formation of the neural tube) in vertebrates. The mechanisms responsible for this movement remain elusive, as neither the way in which epithelial cells exert a tractile force on their substrata nor how cells in a sheet manage to move with respect to one another is fully understood. There are several examples of epithelial movement in early development, in particular epiboly, the spreading of the blastula over the surface of the yolk, and also limb formation in invertebrates, but an especially striking example is amphibian gastrulation.

We have already briefly outlined *gastrulation*, the coordinated deformation of the ball of cells constituting the blastula to establish the basic body plan of the organism. The process of gastrulation is quite complex, comprising *inter alia* cell rearrangement, deformation of epithelial

sheets, and adhesive interactions. Furthermore, in some cases (for example for amphibians) the complicated movement of epithelia moving from outside deep into the interior of the embryo, around an indentation called the blastopore, also forms part of the repertoire of cellular activities contributing to a greater or lesser extent. Gastrulation thus forms an important example of the modes of choreographed epithelial foldings and movements which can cooperate to generate structure; another important model system is *neurulation*, the folding of an epithelial sheet constituting the early development of the vertebrate brain.

The activities of both mesenchymal and epithelial cells are fundamental to developmental processes — chapter 5 gives an extended discussion of aspects of the morphogenetic properties of epithelium and mesenchyme — so that the cellular level, if not the deepest and most basic of the hierarchy, is at any rate the level at which the visible structure formation observed in development occurs. Macroscopic organization cannot always readily be accounted for by more microscopic genetic or molecular properties, as each hierarchical level has its own emergent features arising from the coherent interaction of the subunits at lower levels of the hierarchy. We ultimately seek understanding at all levels.

2.2 Model Experimental Systems

In the experimental and, as we shall see, theoretical study of development, it has proved profitable not to be restricted to the detailed analysis of only *one* organism, but to investigate a number of model experimental systems, species which best display certain features, or which are most readily studied. From the information obtained through such restricted analyses, a composite picture of developmental mechanisms has been built up.

The underlying assumption is that there is a degree of universality in mechanisms across different orders, classes and phyla, so that mechanisms elucidated for one species may be carried over, suitably modified, and yield some understanding for other species. The remarkably conserved genetic code for all living organisms, and the common heritage of species which diversified through evolution, support the assumption that developmental mechanisms are basically similar, and that different animals are on a fundamental level 'variations on the same theme'. In view of the considerable differences between, say, mammals and insects, clearly such a universality assumption cannot be applied uncritically, but must take due cognisance of variations as well as analogies; one problem of embryology is indeed to discover those aspects of the development of a species that account for its unique and characteristic features. Nevertheless, the current state of knowledge provides strong support for the notion that there are a few universal mechanisms that, suitably modified and applied, underly the development of the wide range of existing species.

2.2.1 'Simple' Organisms

Slime Mould

The decision to extrapolate the processes observed in some species to others is also a very practical one, as the ease of experimental manipulation and analysis varies considerably, and it makes sense to extract as much information as possible from those species that most readily reveal

their secrets. An extreme case of this is in the study of the cellular slime mould, *Dictyostelium discoideum*, an organism that alternates between unicellularity and multicellularity, and differentiates into two cell types after aggregation under starvation conditions. The process of its morphogenesis and differentiation displays only the rudiments of development, but it is precisely for this reason that it has become an important experimental system, with the hope of gaining a fairly complete understanding of the mechanisms at work in the absolutely simplest eucaryotic (that is briefly, non-bacterial) case. In particular, the dynamical basis of its development has been modelled on a fairly accurate quantitative basis, and the genetic and dynamical underpinnings of this process have proved to be surprisingly complicated, foreshadowing the complexity of the mechanisms involved in higher organisms. We shall consider slime mould development in some more detail in section 3.3, and a minimal model for the aggregation phase in section 5.1.2 (for an early introductory survey, see the book by Bonner [35]; also see for example [303, pp.203–212]).

Hydra

The small, radially symmetric freshwater animal, hydra, has long been of interest to embryologists because of its remarkable capacity to regenerate a head after decapitation. The regeneration occurs without growth (*morphallactic* regulation), and hydra displays considerable *regulative* capacities, that is, the ability to maintain the correct proportions and patterns over a large range of sizes. The ability to regulate and regenerate has stimulated considerable theoretical interest, and a plausible and experimentally-supported model has been proposed to account for this — features of this explanation are sketched in section 4.2.2. The ability to regenerate in fact correlates with the normal mode of reproduction of hydra, which is by budding, the outgrowth and development of protrusions from the main body column. Thus the studies of normal and regenerative development in hydra proceed in parallel, and amputation experiments reveal much about the normal mechanisms at work (an introductory discussion is given by Gierer [111]).

Nematode

The small cylindrical soil nematode worm (or roundworm) *Caenorhabditis elegans* has become a popular and important research organism in the last few decades, especially in studies of cell lineage. The worm offers many advantages for analysis, including a small generation time (three days to sexual maturity), transparency, a small genome size (about 3000 genes) and in particular an almost perfectly invariant development. The nematode has constant body cell number (exactly 959 somatic, or body, cells in the hermaphrodite, and 1031 in the male; the number of germ cells is not fixed, being about 1000–2000), with a fixed structure incorporating fundamentally the same features, including nerve, muscle, gut and skin tissue, as higher animals. Patient direct observation has enabled the behaviour and lineage of every cell throughout development to be described; the lineage is invariant, arising from coordinated and precisely timed cell divisions, so that the fate of each cell can be predicted from its position in the lineage tree. This is an example of *mosaic* development, with spiral, asymmetrical and determinate cleavage, and no significant ability to regulate.

This careful mapping of each cell activity indicates precisely *what* happens during development, not *how* it happens, but it provides a basis for further mechanistic studies. Many of the

genes have been identified through mutations, and some have been shown to be housekeeping genes which every cell needs to survive and proliferate; some code for 'luxury' proteins that specific cells need to carry out their normal function; while others still are developmental control genes, that direct developmental choices, and whose mutation leads to abnormal lineage and development, such as extra cells or faulty differentiation, disrupting the body plan. Mutations in these genes enable the 'program' and 'subroutines' for nematode development to be identified, and a common underlying mechanism between cell division and cell differentiation is deduced from the fact that these are generally affected in a coordinated way by mutations in developmental control genes.

There are two significant conclusions that have arisen from such studies: firstly, one might have expected that all cells of a particular tissue might be descendants of a single 'founder cell' committed exclusively to a particular pathway. In fact, cells of similar character need not be close relatives, and conversely, very different cells may share a common lineage; for example, in some cases neural and muscle cells are 'sisters'. The other misleading assumption that might arise from the emphasis on cell lineage is that the pattern of divisions and development is cell-autonomous, following an internal 'genetic program'. This is generally the case, but laser ablation experiments, killing off cells and observing the effects on their neighbours, indicate that in some cases intercellular signals and inductive interactions between adjacent cells are crucial to normal development. Thus even an invariantly developing organism such as *C. elegans* displays a complex interplay between internal genetic and environmental factors, so that this effect may be expected to be far more complicated and pronounced in higher animals. A discussion of nematode developmental genetics is given by de Pomerai [77, ch.4].

Sea Urchin

The spherically symmetric, sessile sea urchin, class Echinoida of the phylum Echinodermata, appears at first glance to have little resemblance to chordates, including the vertebrates such as birds and mammals. Nevertheless, it is placed with them into the taxonomic category of *deuterostomes*, as its early embryonic development displays features such as radial and indeterminate cleavage, and the ability to regulate and reform a complete, normal animal from a single cell isolated after the first two cell divisions. The fertilization process in the sea urchin is much studied, but for our present purposes, its pattern of gastrulation is of particular interest and relevance, together with the pattern of signals and determinants that underlies its regional specification. Again its transparency has enabled its internal and external development to be followed and the activities of individual cells to be analysed. The initial fertilized egg is polarized, and animal and vegetal poles may be distinguished. After repeated cell divisions to form the *blastula*, a hollow ball of about 1000 cells one cell thick and surrounding a spherical *blastocoel* cavity, the vegetal pole is the site of the activity that initiates gastrulation.

Briefly, a few dozen *primary mesenchyme cells* detach from the epithelium and enter the blastocoel cavity, where they migrate over its lining, pulling themselves along by extended processes, or filopodia, with sticky tips. Contacts are made and broken as filopodia are randomly extended and withdrawn, until the cells finally appear to settle where adhesive interactions are strongest. Simultaneously, the epithelium at the vegetal pole begins to buckle inward and invaginate towards the blastocoel cavity to form the gut. This process appears to be driven by a change of shape of the affected cells, as well as active repacking, while certain invaginating

cells also extend filopodia into the cavity which adhere to the opposite walls and contract, thus appearing to direct movement and pull the cell sheet inwards (although recent experiments on exogastrulation have questioned this mechanism of filopodial pulling — see section 5.3.2). The outcome of the gastrulation is, as discussed above, the transformation of the spherical blastula into a three-layered structure, corresponding roughly to the basic body layout of the adult, with gut on the inside, epidermis on the outside, and connective tissue in between. Although some aspects of gastrulation are fairly different for other animals — for instance, we have already noted the characteristic epithelial movement around the blastopore occurring in amphibians — studies of the sea urchin have done much to elucidate this vital phenomenon.

2.2.2 Vertebrates

The progression to higher animals, in particular vertebrates, inevitably means that the embryological studies will be considerably more complicated, and the issues arising more intricate, so that we can here only skim the surface of some of the features of vertebrate development, and nevertheless find that our discussions become more lengthy. In the process of these discussions, some of the concepts of experimental embryology will be introduced; for further expositions of these concepts, see [48, ch.43], and especially Alberts *et al.* [1, ch.16] and Slack [327, ch.2].

Amphibians

Amphibians have been studied intensively since the last century, partly as the eggs are large, usually 1–2 mm in diameter, and develop totally outside the mother, thus making them accessible to experimentation at all stages, and especially suitable for micro-operative procedures. The axolotl and the newt have been much studied, historically, but today the South African clawed toad, *Xenopus laevis*, has become for embryological purposes the 'world standard' amphibian for its ease of maintenance, ease of induced spawning and robustness of the embryos. For an introduction to *Xenopus* development, we refer in particular to Slack [327, ch.4].

Early Development The frog egg acquires an animal-vegetal polarity during oogenesis, with a dark pigmented animal hemisphere and a light vegetal hemisphere. The dorso-ventral polarity appears after fertilization, specified by the point where the sperm fertilizes the egg. Both of these polarities apparently involve cytoplasmic localization (that is, inhomogeneous distribution of cytoplasmic components, and a consequent asymmetric cleavage, with daughter cells inheriting different compositions and becoming nonequivalent); a rotation relative to the cell interior of the egg cortex towards the point of sperm entry, associated with the transient appearance of microtubules on the vegetal side, plays a significant role in the dorso-ventral specification. Gravity also appears to play a part in triggering the loss of dorso-ventral symmetry; rotation of the egg is followed by internal cytoplasmic rearrangements in response to the gravitational field. Successive cleavage divisions occur to form the blastula, which is however not spherically symmetric but has larger, more yolky cells at the vegetal end, and multiple layers of cells in contrast to the single layer in the sea urchin.

Regional Specification It is in this blastula stage that the regional specification occurs, and a *fate map* can be established, a diagram to indicate fairly accurately (subject to a small amount of cell mixing which occurs) what each part of the early embryo will become in normal development, and where it will move in the subsequent stages. Such a fate map is created by marking early cells, using vital dyes, or more recently high molecular weight labels or genetic markers, and following their subsequent distribution. Of interest is that some of the cells in the *prospective region* to form future gut, bone and muscle are on the outside of the early embryo; the correct spatial relationships, moving these cells to the inside, clearly arise through the rearrangements of gastrulation. The processes specifying the various regions have come under close scrutiny recently; ultraviolet irradiation and treatment with lithium can have dramatic effects in the early stages, and much is now known about the molecular basis of the inductive interactions. *Induction* is the process whereby chemical signals emitted from one group of cells influence the developmental pathway of another; the ability to respond to inductive signals is called *competence*.

In the case of amphibian embryos, there is evidence that the mesoderm is formed from the animal cap tissue in response to one or more signals emitted from the vegetal region during the blastula stage, whereas in isolation, the entire animal hemisphere becomes ectoderm. The induction is *instructive*, because the responding tissue has a choice before it, and is *competent* to form a range of tissues; but this competence is successively reduced during development, as cells become increasingly *determined* until their fate is effectively fixed. After the formation of mesoderm, the dorsal and ventral regions appear to be differently specified, and a variety of other inductive interactions occurs, in particular 'dorsalization', in which a signal from the dorsal region is required for the formation of *somites* (the precursors of vertebrae and muscles associated with the axial skeleton) from the ventral region. There is increasing evidence that the inducing factors are related to growth factors, in particular to the transforming growth factor beta (TGF-β) superfamily and to fibroblast growth factors; thus here we have a typical example of important proteins playing different roles at different stages in development. For recent reviews of regional specification and induction, see [176, 278].

Gastrulation and Neurulation The significant events of regional specification and patterning in amphibians, establishing the global properties, occur in early development, in the blastula, but as we have seen, the working out of this patterning depends on the morphogenetic movements beginning with *gastrulation*. We have already mentioned the involution, that is the epithelial movement and streaming of cells into the interior (blastocoel), that accompanies the complex motions of amphibian gastrulation to form the primary germ layers.

The next stage of development is called the *neurula*, in which the ectoderm on the dorsal side becomes the central nervous system. The dorsal surface becomes flattened as a keyhole-shaped region, the neural plate, bounded by raised neural folds, which rapidly become more prominent, rise and approach each other, meet and fuse to form the *neural tube*, which becomes covered by the ectoderm form beyond the folds, now known as epidermis. Tissue from the folds which comes to lie dorsal to the neural tube forms the *neural crest*, the cells of which migrate to diverse parts of the embryo to form pigment cells, the skeletal elements of the head, sensory nerves and most of the nerves of the involuntary (automomic) nervous system, and a variety of glands. The posterior part of the mesoderm segregates as a distinct *notochord*, which will elongate and stretch the embryo along its anterior-posterior axis; the mesoderm located on either side of the

notochord becomes segmented, in sequence from the anterior to posterior end, to form the paired blocks of mesoderm called *somites*. Subsequent to the above-described processes of *neurulation* and *somitogenesis*, the rudiments for all the major structures of the body are in their definitive positions.

Induction and the Organizer The process of induction is important throughout development; for example, the optical vesicle, the evagination from the brain that forms the cup of the eye, is required to induce the overlying ectoderm to form a lens (in fact in this case a series of reciprocal inductions occurs for proper development). This phenomenon of signalling between adjacent tissues is best tested by grafting experiments, in which a section of the embryo is transplanted, and the subsequent development studied; the graft might respond to inductive signals from its new location, or itself emit signals that alter the developmental pathway of its new environment.

The most dramatic and famous example of induction derives from the *organizer* experiment, originally performed by Spemann and Mangold on newt embryos (1924). A piece of tissue from the dorsal lip (of the blastopore region, past which epithelial layers of cells stream into the cavity) at the early gastrula stage, was transplanted to the opposite side of the embryo, with the result that a complete second embryo formed at the site of the graft. The dorsal lip, called the *organizer* by Spemann because of its primary role in the early stages of organogenesis, is responsible for setting up the interaction between the ectoderm and mesoderm; so that its transplantation induced a secondary invagination and gastrulation, and in particular a second nervous system from the part of the ectoderm that lay above the organizer subsequent to gastrulation. Thus the host tissues had completely changed their fate, and a complete new embryo was created. Such neural induction is in fact a crucial process of normal development, with the nervous system formed as a result of a 'neural inducing factor' signalled from the dorsal mesoderm.

The organizer experiment was the first unequivocal demonstration of cell-to-cell interactions, which were shown to be due to a chemical signal when it was found that the induction could take place even if the organizer tissue were killed. The intensive search for its biochemical nature fizzled out, however, when it was found that many signals, ranging from rat liver to heat shock, had the desired effect. In contrast to the instructive induction specifying mesoderm described above, neural induction is *permissive* and non-specific, serving only to trigger off a developmental pathway; the ectoderm is delicately balanced between epidermal and neural pathways, and a variety of stimuli can tip the balance one way or the other.

The above brief outline of the early stages of amphibian (more specifically, *Xenopus*) development has introduced many of the major issues and concepts in general vertebrate development, including the blastula, gastrula and neurula stages, and the importance of cytoplasmic localization, intercellular signalling and inductions. Before we proceed to touch on bird and mammal development, it is useful to consider another feature of some amphibians, not directly related to development but important in the consideration of patterning mechanisms, namely regeneration.

Regeneration The classic regeneration experiments have been performed on the newt: when this animal loses a limb, the wound heals over, and there is a gradual accumulation of the *blastema*, a mass of cells which derives from muscle, bone and other differentiated cells which have dedifferentiated and regain the potency to form the variety of limb cells, thus acting much

like a limb bud. The blastema at the tip then grows and differentiates into the specialized tissues of the limb, forming the structure that was lost; in contrast to hydra discussed above, regeneration is by growth (*epimorphic* regeneration) instead of by rearrangement of existing cells (*morphallactic* regeneration). The amount of limb regenerated corresponds correctly to the level of amputation, giving rise to questions as to how the cells 'know' what structures are present in the limb. Further cut and graft experiments have posed more conceptual problems, on account of the sometimes surprising results occurring, such as the creation of additional limbs, when say a right limb is grafted onto a left limb bud.

The mechanisms of regeneration, and in particular the specification of pattern, are of interest as they may be assumed to be related to the embryonic mechanisms utilized in normal development, and we shall discuss a model that has been proposed for regeneration in newt limbs (together with the analogous phenomena observed in cockroach legs and insect imaginal discs) in section 4.2.6.

Chick and Mouse

We have considered at some length the early stages of development of amphibians. The motivation for much embryological study is the understanding of our own origins, and the obvious ethical difficulties associated with experimentation on humans have led to the search for organisms that would serve as models for human development. The two that best appear to satisfy this criterion and are hence much studied are the chick and the mouse. As our goal here is not comparative embryology, the similarities and differences with frog development will not be elaborated here, but only some brief matters of interest pointed out; for a fuller discussion, see Slack [327, ch.6].

Features of Mouse and Chick Embryology The embryos of reptiles, birds and mammals are unusual in the animal kingdom as, instead of developing in isolation, they are nourished by the yolk of the egg (for birds and reptiles) or the maternal blood stream (for mammals), and thus undergo a considerable amount of growth during development. A feature of these classes is also the formation of extensive *extraembryonic membranes*, the amnion, chorion, allantois and yolk sac, that mediate the nourishment of the embryo and form a substantial proportion of the cells in early development. These adaptations to the mode of embryonic life of these animals are responsible for considerable differences in the morphology of the early stages, and in the gastrulation movements, compared to amphibians, but the resultant primitive body plan obviously resembles that of the frog, and the mechanisms of induction and some biochemical signals that have been elucidated are homologous to the corresponding aspects of amphibian development.

The chick is the most popular avian embryological experimental system. It has the clear advantage that the embryo is accessible at all stages following cleavage, *in vitro* culture of early blastoderms is possible for long enough to form a recognizable body plan, and grafting experiments are readily carried out. Thus our knowledge of the normal morphogenetic movements, the fate map, early determinative events and inductive interactions is reasonably good. The mouse, on the other hand, suffers from the same experimental disadvantage as other mammals, namely that it is viviparous, and thus the development is inaccessible following implantation into the

uterus. *In vitro* studies are only possible for the early stages of development, so that mouse embryology is really 'pre-embryology', dealing with the formation of the various extraembryonic membranes necessary to the support and nutrition of the embryo, rather than the embryo proper. In order to circumvent this problem, a variety of novel investigative techniques has been used, including the culturing of embryonic stem cells and teratocarcinoma cells, which can be retained in an undifferentiated state indefinitely. Chimaeric mice, those resulting from a mixture of cells from different zygotes, have also been studied with a view to the understanding of cell sorting, in particular.

Genetic Analyses Extensive genetic analyses have also been performed on the mouse, largely using oncogenes (cancer-inducing genes) which frequently have vital developmental functions, and also genes homologous to those important in early development in *Drosophila* (see below). The creation of *transgenic* mice, those with an altered genetic constitution, in order to attempt to study the functions and regulation of the modified genes in normal development, has also recently become popular. Such studies have indicated considerable similarities in the genetic basis of the development of animals as diverse as mammals and insects; many genes, including some carrying the homeobox, a highly conserved sequence coding for a DNA binding domain (see the discussion of *Drosophila* development), and others with a 'zinc finger' — homologous to transcription factors in *Xenopus* — or growth factors, are being studied, and appear to have important functions in embryonic development. Particularly intriguing are the "homeobox homunculi" [159, p.64], clusters of homeobox genes for which the anatomical order of expression, in terms of anterior boundaries, is the same as the spatial order in which the genes are arranged on the chromosome within each cluster. The functions of these genes are not known, but a popular view is that different combinations of gene activity are the epigenetic codings for different anterior-posterior body levels — see [78, 278].

In the studies of early development of the mouse and chick, there has been less discovery of *new* mechanisms as inquiry into how the mechanisms elucidated in other experimental systems, such as *Xenopus* or *Drosophila*, are manifested in birds and mammals, and by extrapolation, in humans. Later developmental stages of the chick, in particular, have been of considerable interest in their own right, however. The chick has long been a favoured organism for the study of the migration of neural crest cells, and integumental (skin) patterning (see section 5.1.1), but of especial interest is the developmental patterning of the chick limb.

The Chick Limb The embryonic chick limb provides one of the best model systems for analysing how an organ develops once the body plan is laid down: it is easily accessible to experimental manipulation, and the basic pattern of cartilage elements is quite simple compared to the often convoluted and intricate structures of other organs. The wing pattern, along the proximo-distal axis (that is, measured from the point of attachment to the body to the tip) comprises the main element, the humerus, followed by two smaller elements, the radius and ulna, then some smaller structures in the wrist and finally three digits; a similar pattern exists in the leg. This development occurs relatively late, once the basic body plan is already well established, and features of the head such as the eye are already prominent; but the limb constitutes an independent developmental system (or *field* — see section 5.3.4) which may readily be manipulated to investigate the patterning mechanisms. The outgrowth of the limb begins with the formation of a small bulge of cells, the limb bud, with a thick structure, the *apical ectodermal*

ridge — the presence of which is necessary for growth — at the tip. With cell multiplication, the bud grows out in a paddle-like form, and soon the cell density in the core increases to form an aggregation of mesenchymal cells, which will become the cartilaginous rudiments of the humerus, later differentiating into bone. With further growth, the cell condensations corresponding to the radius and ulna develop, then the wrist elements and finally the digits.

Numerous experiments have been performed to establish the nature of the decision-making process that leads to this reproducibly patterned sequence. The results of these studies are best presented in the light of theoretical models that have been proposed to account for the observations, with considerable success, so that we shall not consider them in further detail here, referring instead to sections 4.2.4 and 4.2.5. Briefly, two regions in the embryonic limb are found to have a seemingly determinative effect on the patterning: the apical ectodermal ridge is essential for outgrowth and the correct sequence along the proximo-distal axis, while a small group of cells at the posterior (rear) margin of the limb bud, known as the polarizing region or *zone of polarizing activity* (ZPA), appears to control anterior-posterior patterning, as the pattern is dramatically modified if it is transplanted. Considerable progress has been made in the investigation of the nature of the signals involved, but discussion of this is postponed to chapter 4, where it is placed in the context of appropriate theoretical paradigms (see also the popular articles by Wolpert [376, 379]).

The mouse and the chick, in embryological studies the closest 'models for man' [327, ch.6], are sure to play an increasingly important role in future investigations, as genetic insights gained from other organisms, notably *Xenopus* and *Drosophila*, are transferred and adapted to these amniote vertebrates, and in particular with the development of improved methods of post-implantation study in the mouse. The current state, however, still sees the chick limb patterning studies as possibly the most important novel contribution these organisms have made. The final experimental system we shall consider, *Drosophila*, has already been referred to, and is presented here last, out of taxonomic order, on account of the extensive and fascinating progress that has been made in approaching true understanding, on a genetic level, of the patterning mechanisms involved in establishing the body plan.

2.2.3 *Drosophila*

The past decade or so has seen a massive explosion of interest and significant results in the molecular genetics and early regional specification of the fruit fly *Drosophila melanogaster*, with the result that it is now clearly the best-understood developmental system, in terms of the early decision-making processes. Indeed, a satisfactory explanation of the mechanism and genetic controls determining the early developmental program is now being attained, with the regulatory genes that control patterning in the early embryos being identified and their role in patterning and the regulation of other genes elucidated, while several significant gene binding domains, including in particular a highly conserved sequence known as the *homeobox*, have been discovered. While these studies have revealed little about the detailed morphogenesis of the final organs, with its cellular movements and interactions, they have revealed a fairly comprehensive picture of the specification of the overall body plan.

As before, we can only sketch a brief outline of *Drosophila* development; for a nontechnical discussion, see [378, ch.VII], while for more detailed introductions, see [1, pp.920–940] and [327,

ch.7]. Comprehensive recent reviews of this rapidly changing field are given by de Pomerai [77, ch.5], and especially in the attractive book *The Making of a Fly: The Genetics of Animal Design* by Lawrence [200].

Overview of Development The egg of *Drosophila*, a species of fruit fly, has an elongated ellipsoidal shape, with a well-defined polarity. After fertilization, development begins not by proper cleavage, but by a series of nuclear divisions without cell division, creating a *syncytium*. The early nuclear divisions are extremely rapid, and plasma membranes develop between the nuclei only after 14 divisions, at which stage there are about 5000 nuclei. The basic ground plan that we shall describe, with the characteristic segmental gene expression pattern, has already been laid down by this stage. The subsequent development of the multicellular embryo incorporates the movements of gastrulation, and after about a day a segmented feeding larva is formed. The larva passes through three stages, or *instars*, separated by moults. At the end of the third instar it pupates, undergoing complete metamorphosis, and eventually, about nine days after fertilization, an adult fly or *imago* emerges.

Fundamental to the structure of the fly is the *segmentation pattern*, the modulated repetition of a basic segmental unit, though with each segment having its own identity and characteristic features. This pattern of 14 divisions (it is frequently convenient to consider 14 *parasegments*, half a segment out of register with the traditionally defined segments but corresponding more closely to the gene expression periodicities) is even more marked in the larva, and the major goal of *Drosophila* studies, one that has largely been attained, is to establish how this segmentation pattern is controlled and laid down.

Segmentation and Hierarchies of Gene Expression

Recent breakthroughs in genetic studies have largely established the details of the hierarchy of developmental decisions, concerning the sequential activation and control of regulatory genes, which lead to the specification of the segmentation pattern and the assignment of unique patterns of gene expression to each segment. In the present brief description of this regulatory hierarchy, we shall have to preempt some of the theoretical concepts presented later, on the grounds that they now appear to be experimentally established; nevertheless, the discussion of the concepts of coordinates and gradients in chapter 4 will proceed from anew, without regard to these preliminary indications of their relevance. More details and references concerning the outline below may be found in any of the above works; for a recent review on *Drosophila* pattern formation, see [314].

The Dorso-Ventral Axis The *Drosophila* pattern is laid down independently along two orthogonal directions, the dorso-ventral ('top-bottom') and anterior-posterior ('front-back') axes. Effectively, one can speak of two orthogonal coordinate systems needed to define each position in the blastoderm. A group of about 20 genes controls the dorso-ventral axis, so that mutations in any of these genes give rise to embryos that are either 'dorsalized' and lack ventral structures, or 'ventralized', lacking dorsal structures. In particular, events in the egg chamber of the mother lead to an inhomogeneous distribution of the product of the gene *dorsal*, which is distributed in the nuclei of the blastoderm in a dorso-ventral concentration gradient. It is thus

considered a *maternal-effect* gene, with the mRNA and hence the phenotype deriving from the maternal genome, and the inhomogeneity being present in the egg before fertilization (although the mRNA, localized in the zygote, is only expressed after fertilization). As it plays a role in the establishment of primary polarity, it is also termed an **egg-polarity** gene. The dorso-ventral system is responsible for the formation of mesoderm, the presumptive nervous system and epidermis.

The Anterior-Posterior Axis — Egg-Polarity Genes Of greater interest and complexity is the anterior-posterior system, in which the segmentation occurs. The egg has an initial polarity along this axis also, depending on the distribution of materials produced before fertilization; and again mutations in the egg-polarity genes cause disturbances in the anterior or posterior parts of the body. Thus mutations in the gene *bicoid* produce embryos lacking head and thoracic structures, while the egg-polarity mutation *oskar* gives rise to embryos lacking all the abdominal segments. These genes form the first level in a hierarchical system that generates the anterior-posterior pattern of the body. More generally, three maternal systems, the anterior system exemplified by the gene *bicoid*, the posterior system with the gene *nanos*, and the terminal system containing the mRNA of the *torso* gene, are established; the products of these three systems divide the embryo into several zones depending on their concentrations. Of most interest, the anterior cytoplasm of the zygote contains maternal mRNA for synthesizing the protein encoded by *bicoid*; soon after fertilization, this protein is produced, and diffuses down the syncytial blastoderm, in the absence of cell membranes, to produce a concentration gradient that guides the global organization of the anterior half of the embryo.

Segmentation Genes The graded global cues provided by the egg-polarity genes have to guide the creation of a system of discrete segments; this process depends on the **segmentation** genes, which fall into three classes. The first to act are the **gap** genes, whose products mark out the coarsest divisions of the embryo. They are expressed in particular regions, usually covering a few segments, based on the concentrations of the egg-polarity genes; the best-characterized of these genes are *hunchback*, *Krüppel* and *knirps*.

The next segmentation genes to act are the **pair-rule** genes, such as *hairy*, *even-skipped* and *fushi tarazu*. These are expressed in stripy patterns with a periodicity of two segment widths, but the different genes differ in their precise expression patterns with respect to the segmental or parasegmental borders. Their expression is determined by the gap gene products, which regulate finer details of patterning than the egg-polarity genes; their periodicity arises because many different combinations of maternal and gap genes can activate the same pair-rule gene.

Finally, there are at least 10 **segment-polarity** genes, which are expressed in a similar portion of every parasegment and thereby label its basic subdivisions. Most important are the genes *engrailed* and *wingless*, whose expression is in a repeating pattern of single segment width periodicity, and causes the subdivision of the axis into parasegments. Reciprocal interactions between these gene products stabilize their expression and more clearly define the differences between different parts of the parasegments, so that the correct expression patterns depend on mutual interactions and feedback controls between gene products at a particular level of the hierarchy. Thus we have seen that each position along the axis is uniquely specified by the expression of a particular combination of gap, pair-rule and segment-polarity genes, and

a hierarchy of regulatory interactions exists to produce successively finer patterning through sequential subdivision of the syncytium.

Homeotic Selector Genes The segment-polarity genes generate a lasting record of the relative positions within each segment, but the egg-polarity, gap and pair-rule genes, though providing each segment with a unique identity, have transient patterns of expression. The distinctions between segments or parasegments are maintained through the differential expression of **homeotic selector** genes. These are expressed in a unique combination in each segment due to its particular pattern of segmentation gene expression, and hence give each segment a specific 'molecular address label' providing a 'memory'. The character of each segment is determined by the homeotic genes expressed, which thus control its subsequent pathway of differentiation. The homeotic genes are largely found in two gene clusters, each containing several genes with analogous functions, the *bithorax complex* and the *Antennapedia complex*. The bithorax complex genes control the differences among the abdominal and thoracic segments of the body, while those in the Antennapedia complex control the differences among thoracic and head segments.

Homeotic genes were first identified through homeotic mutations, those which have drastic effects causing the replacement of one fly structure by another. For example, mutation in the gene *Antennapedia* leads to the replacement of the antennae by second, or mesothoracic, legs. Such homeotic mutations are readily accounted for in the light of the function of these genes in normal development; essentially, if homeotic genes provide each segment with a unique identity, then a mutation causes the segment to be 'misinformed' about its identity, and thus to make a structure appropriate to another region. Homeotic genes thus form the lowest level in the five-level hierarchy of genes that controls primary patterning and thus the developmental pathway to be followed, essentially independently within each segment, by the embryo.

The Homeobox

The homeotic genes endow each segment with a permanent label, and control the coordinated expression of many other genes to create the structures characteristic of that segment. Thus they must play an important regulatory function in promoting or repressing the expression of other genes. A molecular basis for this function is provided by the *homeobox*, a DNA sequence of about 180 nucleotide pairs that is contained, with minor variations, in virtually all the homeotic selector genes and certain other genes (such as the egg-polarity gene *bicoid*, the pair-rule gene *fushi tarazu*, and the segment-polarity gene *engrailed*). The presence of this highly conserved sequence in so many important genes, which generally code for proteins located in the cell nucleus, suggests direct involvement of the homeobox in the control of gene expression and hence a significant role in patterning. All the evidence suggests that the homeobox codes for a specific DNA binding domain, which underlies its regulatory activities (other DNA binding motifs, such as zinc fingers, are also common).

Genes containing a homeobox tend to be involved in regional specification or play an otherwise significant role in patterning. This has been singularly useful to developmental biologists, who have used this conserved sequence as a probe to search for formerly unknown genes which may participate in early developmental patterning. Such a search has been extremely fruitful, as the homeobox has been found to be highly conserved during evolution, and homeobox-containing

genes have been found in nematodes, sea urchins, and in vertebrates including frogs, chickens, mice and humans. Although these genes in other organisms have not been shown to have as fundamental roles as those in *Drosophila* — in particular, such drastic homeotic mutations have not yet been detected — some have been found to be involved in patterning. The considerable conservation of the sequence over millions of years of divergent evolution has led to hopes that there might be fundamental similarities between insects and vertebrates in the mechanisms that control the development of the body plan; as we have noted above, such considerations and analogies have for instance motivated much of mouse embryology.

Of particular interest is the fact, not yet accounted for, that the spatial pattern of expression of the homeobox genes of a gene complex in the fly embryo corresponds to the spatial order of the genes on the chromosome; a similar situation obtains for the expression of homeobox genes along the main body axis of the mouse, and in the chick limb (see section 4.2.4). This raises an intriguing possibility that the homeobox genes may in some way be molecular markers of position, and that there was a correlation between the spatial sequence on the chromosome of their expression, at least in early stages of evolution, and the regions in which they were expressed. Nevertheless, in spite of the clearly developmentally important features of the homeobox and homeotic genes, it must be borne in mind that these constitute only *one* aspect of a complex, tightly coordinated and regulated hierarchy of gene expression patterns that establishes the primary pattern of the *Drosophila* embryo. For early introductions to this stretch of DNA that has excited so much interest, see [108, 107], while [226] is a more recent review.

Imaginal Discs

The patterning processes we have considered have referred largely to the larval segmentation, but many of the fly structures, such as legs, wings and antennae, only become apparent after metamorphosis — how are early misleading instructions retained to give rise to the dramatic homeotic mutations in the adult structures? The adult fly is formed largely from groups of cells, called *imaginal discs*, which originate from embryonic epidermis and are set aside, apparently undifferentiated, in each segment of the larva. Thus there is a pair of discs for the eyes and antennae, another for the first pair of legs, another for the wings and part of the thorax, and so on. Although the cells of the imaginal discs look essentially like one another, grafting experiments demonstrate that they are already regionally determined and nonequivalent. The homeotic selector genes play a crucial role in this determination, and the imaginal disc cells retain the memory of their determination through the many cell cycles that fall between the initial expression of homeotic genes and the formation of adult structures after metamorphosis, by mechanisms such as positive feedback, whereby the homeotic protein products act to stimulate their own gene transcription, and heritable changes in chromatin structure.

A significant feature is the discreteness of the choices: there is an abrupt difference in gene expression in adjacent parasegments, due to different combinations of homeotic selector gene expression (a similar situation prevails for the distinction between posterior and anterior parts of the parasegment through differential expression of the segment-polarity gene *engrailed*). At the frontier between one such region and the next, cells with different 'address labels' appear to be prevented from mixing. For instance, if a clone of genetically marked cells is created in the wing, that clone is observed to be confined strictly to one side or the other of a precisely specified boundary marking the frontier between the two parasegments from which the wing is

constructed. Such a subdivision of the body or an organ thereof is called a *compartment* (see section 4.3.3); although the boundary does not necessarily correspond to any visible structural feature, it does coincide with the domain of expression of homeotic selector genes and *engrailed* gene expression.

We have dealt with the hierarchy of decisions characterizing the early developmental patterning and regional specification of *Drosophila* at some length, as it is currently the best-understood system by far, in terms of the genetic underpinnings of its development. The information gained here has revolutionized our knowledge of how genes control development, and the insights gained are being applied to other systems, including vertebrates, with far-reaching results. We shall see many of the themes presented here, including coordinate systems, gradients, and stripes of segmentation, recapitulated in the theoretical sections of this thesis; for many of the concepts that have long been proposed, *Drosophila* provides the first concrete experimental support.

Several experimental systems have been discussed, each of which has made its own peculiar contribution to the understanding of developmental mechanisms; in terms of value and influential ramifications of the information gained, and interest and excitement stimulated among embryologists, *Drosophila* must surely rank very near, if not at, the forefront. Nevertheless, the current status of knowledge is only the 'tip of the iceberg', and needs to be built upon extensively, with the aid of intensive and innovative experimentation and pertinent theory, possibly such as that to be developed in the remainder of this thesis, in order to approach a reasonably complete understanding of developmental mechanisms accounting for the formation, ultimately, of ourselves.

A Note: the Emphases of our Discussions

It will be apparent that the above discussions of particular model experimental systems have dealt largely with early embryonic development, and in particular pattern formation and regional specification in the early embryo. This is somewhat in contrast with the theme of the previous section 2.1, where the morphogenetic properties of molecules and cells were considered, with respect to the later shaping of tissues and organs. The reasons for this apparent discrepancy are clear:

The basic patterning effects of genes are best elucidated under the relatively simple conditions of early development, where there is less structure, so it is easier to see what is going on, and manipulations are also more readily achieved. Concerning the overall patterning process, the laying down of the global body plan is crucial, while the rest of the patterning is, to some extent, refinement. Furthermore, we would expect the establishment of later axes and regional differences to be less of a conceptual problem for the finer detail, when so much local structure already exists, and localized interactions can presumably account for the fine-tuning through for instance local cell-cell signalling, growth or deformations, than for the early patterning where regional differences and asymmetries have to be created in a largely featureless, virtually homogeneous environment.

On the other hand, many of the morphogenetic mechanisms utilizing the diversity of properties of mesenchymal and epithelial cells and the cytoskeleton, CAMs and the ECM, only really come into their own in later developmental stages, when the organs are moulded from differentiated tissues. Ultimately, we expect the developmental mechanisms to be available throughout

development, with some playing a greater role in earlier and others in later stages, so that it is appropriate to study them where they are most applicable. The theoretical approaches we shall consider deal with both aspects, early embryonic pattern formation and later morphogenetic processes in organogenesis.

2.3 Some General Comments

The above model experimental systems and the repertoire of genetic, molecular and cellular mechanisms that have been outlined provide the experimental 'raw material' with which our theoretical concepts and models are concerned. The features described above thus circumscribe the domain of interest for the present study, and it will be noted that in the overall context of developmental biology, this domain is not nearly as general as it could be. Some of the exceptions will briefly be accounted for below; clearly, limitations have to be placed on a work which already has as broad a scope as this.

2.3.1 Plant Development

Possibly the most major limitation to our study is the restriction to animal development — together with some unicellular protists, such as *Dictyostelium* (section 3.3) and *Acetabularia* (sections 4.3.4, 5.2.2), which form simple model systems able to illuminate some mechanisms of animal development. Plant developmental biology is an entire field of study in its own right, with many similarities to animals but also its own characteristic features, which could not be considered here.

In particular, the presence of a cell wall around plant cells, providing structure and rigidity, constrains plants to strategies for reproduction, growth and development that are quite different from those adopted by animals. Thus spatially regulated cell differentiation is utilized by plants as well as animals, but whereas animals make extensive use of migrations, deformations and reassortments of cells, oriented cell divisions and strictly regulated cell expansions are crucial to the morphogenesis of plants. Furthermore, while the plant also passes through a characteristic embryonic stage in the seed, in which the adult structures are moulded from an embryo looking nothing like the adult plant, the addition of new cells at particular regions (meristems), growth and morphogenesis occur throughout the life of the plant. The pattern-forming potentials of many of the mechanisms of plants are thus not as restricted to a particular developmental stage as those of animals — especially as many differentiated plant somatic cells retain the ability to regenerate whole plants — and the resultant structures formed are very different, so that we might expect considerable differences between plant and animal developmental mechanisms. The study of plant development is not nearly as well advanced as that of animals, although extensive tissue and cell culture experiments have been performed, and genetic studies, especially with the weed, the common wall cress *Arabidopsis thaliana*, are beginning. For an introduction to plant development, see for example [1, ch.20].

2.3.2 The Nervous System

Aside from essentially neglecting plant development, we also ignore the question of pattern formation in the nervous system in this work. The nervous system is the most ordered, organized piece of matter known, and its development the most intricate assembly process of all. The problem is not so much the standard issue of developmental biology: how do the different cells arise, differentiate and come to be arranged in their proper places? These questions may be answered for nerve cells in terms of the same principles that apply to other cell types. The nervous system instead poses an additional problem: how do the nerve cells come to be properly connected? For it is the vast number of these complex and regulated connections, arising from the orderly outgrowth of axons and dendrites and the formation of a regular system of synapses, that determine the proper functioning of the brain, and must ultimately provide the anatomical and physiological basis for consciousness. Of particular interest is the dichotomy and apparent tension between, on the one hand, *random* synaptic connections which are constantly released and remade based on their firing rates (constituting 'experience'), and on the other hand, the very *precise* wiring that must exist, for example to prescribe reflexes and behavioural patterns that appear fixed and 'hard-wired'.

The field of brain and neural structure, function and development, incorporating the theories that have been proposed to account for these mysteries, is vast, and no attempt will be made here even to touch on the issues involved. For a preliminary introduction to the problem of 'wiring the brain', see Wolpert [378, ch.VIII] and the article by Kalil [177]; other introductions are given in the general treatments of Gilbert [115, ch.17] and Alberts *et al.* [1, ch.19]. Numerous more detailed and specialized treatments exist; for two very different approaches to the 'matter of the mind', the books by Penrose [305] (who does not, however, consider the question of development in any depth) and Edelman [83, 85] are recommended.

2.3.3 Information

The discussions in this chapter of some aspects of development should have made it abundantly clear that the statement 'DNA controls development' is a complex one, to be considered with care. On one level, sequential and coordinated gene expression directs patterning processes, such as in the early regional specification of *Drosophila*. On another level, however, the effect of genes on morphogenetic phenomena is both poorly understood and very indirect. The molecular and cellular behaviours, such as movement and cell sheet deformation, that constitute the formation of biological structure, are macroscopic effects that are hierarchical levels of organization above the genes, though ultimately deriving from the genetic 'instructions'. It is the interplay between *genetic factors* and *macroscopic contraints*, such as energy-minimization conditions, that permits pattern formation and morphogenesis. In short, even complete knowledge of the DNA sequence of an organism would enable us to predict almost nothing about its final structure and the developmental processes that led there.

There has been considerable confusion about the status of DNA as a 'controlling influence' in development. The issues have revolved around whether 'genes control' development, whether the genome embodies a 'program for development', or whether 'developmental information' is encoded in the genome in some way — and if so, whether the DNA contains 'sufficient' information to account for developmental processes, or whether some other, higher, guiding

principles or constraints need to be invoked or introduced. No serious attempt will be made here to deal in any way adequately with these issues — the most appropriate response is, indeed, the study of developmental mechanisms that manifest macroscopic constraints at a higher hierarchical level, and are logically irreducible to any analysis in terms of genetic 'programs' or 'information'; and it is this study that will occupy us in this thesis. Nevertheless, a few brief comments on 'DNA as information' are given below, and section 6.2.3 contains some remarks on the 'genetic program' concept and the application of algorithms and rules in development. Useful discussions of the role of genes in development, and the status of some of the metaphors that have arisen, are given by Nijhout [280] and Goodwin [125].

DNA as Information

The concept of 'information' is used with two very different meanings in biology [280]. Firstly, the sequence of bases in DNA *codes* for the sequence of amino acids in proteins, by means of the universal genetic code assigning a unique amino acid to each codon triplet. In this restricted sense, the DNA *does* contain information, namely about the primary structure of proteins. It is when this perfectly valid concept is extrapolated to the second interpretation of 'information', used to imply that the genome hence somehow 'codes' for the complexity of animals, that matters are somewhat obscured.

There is no one-to-one correspondence between the base sequence and the final intricate and functional structure of organisms. The genome by no means contains sufficient 'information' to specify the coordinates and states of each of the cells (of the order of 10^{13} in humans) in the final organism individually, but this is not required — genes act through the hierarchy of determinative patterning and morphogenetic events, and structures and forms are moulded and refined by the interplay between cells using physical and chemical interactions and signals, without necessarily any genetic input. Thus it makes no sense to consider the 'information content' of the DNA without understanding the mechanisms by which the genetic instructions specify the formation of a multicellular organism. This is because these mechanisms frequently constitute higher levels of organization, epigenetic factors, without which the correspondence between genotype and phenotype cannot be established.

The situation may be likened [124] to one in classical mechanics: form correlates with genotype as the orbits of bodies under the action of central forces correlate with initial conditions. A knowledge of the initial conditions alone will not allow us to predict the motion of the particle; the laws governing its behaviour are also required. Similarly, morphogenetic 'laws' or mechanisms are crucial in the understanding of the formation of forms on the basis of the genetic information.

As evidence that the length of the genome (which on a simple 'bit-count' interpretation, must correlate directly with the information content) is no reasonable measure of the complexity of the resultant organism, we may consider that only about 5% of the DNA is translated into protein; the rest consists partly of introns, which are spliced out of the messenger RNA (mRNA) and seemingly discarded, but mainly of non-coding DNA corresponding to regulatory sequences such as promoter and repressor regions, and simply to 'junk'. Also, amphibian DNA contains more nucleotide base pairs than that of mammals — largely to buffer the biochemistry of the developing frog against temperature changes in ponds — and some amoebae contain a hundred

times as much DNA as either. Viewed in this way, the concept of DNA as pure information, correlated with the complexity of the resulting organism, must be flawed [63].

In classical information theory the encoding/decoding method is just as important as the actual message, and this realization provides the resolution to our problem: in the 'transmission' of genetic 'information' from parents to offspring, the *context* in which the genetic 'message' is interpreted is crucial [63, 280]. In the absence of an appropriate interpretive mechanism, no amount of genetic information can produce structure, whereas in the correct context, it is possible, as we shall discuss at length, that very little 'information input' is needed to trigger off an extensive, autonomously proceeding sequence of patterning events. The context required for development includes the correct intracellular, cytoplasmic organization and machinery, to permit the correct transcription and translation of the DNA, as well as more global mechanisms used to produce form and structure in morphogenesis, that is 'generic' physical mechanisms as opposed to 'genetic' factors [268], arising from the cellular properties outlined above (also discussed later, in chapter 5). Without due regard for such a context, the relationship between gene activity and actual development may indeed appear illogical [203]; this is simply an expression of the fact that developmental processes may *not* be reduced merely to a 'genetic program', and that organismal complexity is irreducible to pure 'genetic information' [125].

Self-Assembly An important illustration of autonomous structure formation, demonstrating biological processes that occur independently of direct genetic input, is given by the process of *self-assembly*. The three-dimensional structure of proteins and viruses is formed autonomously once the one-dimensional primary amino acid sequence is created, with the shape determined by the energy-minimizing configuration (based for example on preferred bond angles) — all the 'information' required for assembly is built into the molecules themselves. For example, the bacteriophage T4 has a perfect icosahedral shape, but the representation of this structure, arising from the bonding properties of the constituent protein molecules, is distributed over many genes in the viral DNA; there is no gene in T4 that specifies "make an icosahedron" [203]. By extrapolation, one can imagine that "the more complicated anatomies of multicellular organisms are built skyscraper-style from so many intermediate levels of interactions — each with its own emergent properties — that the edifice is virtually epigenetic, soaring far above its genome" [159, p.64].

In the formation of cellular organization, self-assembly processes analogous to those observed in molecules may also be assumed to operate. Once the initial conditions are given, and the process has started, the dynamic properties of cells and the constraints imposed by the properties of the membranes and environment determine the final structure [16]. The role of the genes is to provide the initial conditions for morphogenesis and to trigger the process, which then proceeds autonomously and predictably from the cooperation of mechanical forces and geometric constraints. Such self-assembly mechanisms, deriving from the morphogenetic properties of constituent cells and tissues, are unable to account for significant aspects of development, such as primary patterning, but they do give an indication of how development can also proceed epigenetically, without need for constant genetic instructions beyond establishing the initial cellular properties. Two features of such self-assembly should be noted, however: it requires pre-existing structure and inhomogeneity, as an initial condition for the action of the participating physical processes; and it is driven by the inexorable tendency towards equilibrium and an energy-minimizing configuration.

For most of the remainder of this thesis, we shall consider, in contrast to self-assembly, the pattern-forming, morphogenetic properties of *self-organization*, or symmetry-breaking processes, which may account for the *initial departure* of the system from homogeneity, and the *spontaneous generation* of structure and form. To begin with, we consider self-organization on a physical and chemical basis, placing it in its wider context, with no direct reference to biological development; while in subsequent chapters, the connection between the physical concept and the experimental results presented here will become apparent and be elaborated by means of numerous examples of pertinent models of self-organization in biological development.

Chapter 3

Self-Organization

In the study of pattern formation and morphogenesis, the development of biological structure and form, a crucial question is how a structure that appears initially symmetrical and homogeneous can 'spontaneously' generate asymmetry and inhomogeneity — that is, *self-organize*. In the strict sense the paradigm of self-organization may frequently be an idealization, inappropriate to many developmental situations, as will become apparent at various points throughout this thesis; most pattern formation does not occur from a homogeneous basis as a response to purely random perturbations, and biological systems are 'driven' by external thermodynamic forces, such as temperature and chemical gradients, which may impose asymmetries. Nevertheless, the concept of epigenetic pattern-forming mechanisms, related to the feedback activation and inhibition processes of, in particular, chemicals and mechanical forces far from equilibrium, and the concomitant generation of 'dissipative structure' in a driven system, has proved to be, at the very least, a productive metaphor, with which we shall be concerned in detail in this work.

A motivation for the present account of self-organizing mechanisms is that the concept of self-organization, which as we shall see appears as a basic philosophical assumption of a wide variety of models, is frequently not made explicit, and is hence not placed in its wider physical and chemical context (with some notable exceptions, for example in the work of Goodwin [126], Haken [141] and especially Prigogine and the members of the Brussels school [10, 276, 277]). We wish here to give an account of that context, as the conceptual framework within which a fundamental unity may be seen to underly a diversity of developmental situations.

Before we can proceed to furnish a proper and balanced analysis of the extent of usefulness and validity of the concept of self-organization in development, we must thus consider in more detail the notion of self-organization, "the spontaneous generation of complexity" [28, p.137]. In so doing we take cognisance of the fundamental paradigm shift that has occurred in some physical sciences over the last few decades, in the recognition that organization and complexity *do* fall within the ambit of physical explanation; and that complex structures and processes must not *necessarily* result only from complex causes or initial conditions.

3.1 Self-Organization and Complexity

3.1.1 Nonlinear Systems as a new Frontier of Physics

Biological systems are physical systems. This is not to say that biology is the same as physics — the objects of biological investigation are unique in their complexity and functionality of structure, hierarchical organization and competence for apparently purposeful behaviour — but that they are nevertheless composed of the basic building blocks of matter, atoms and molecules, or ultimately quarks and leptons, and that their components obey the same fundamental laws elucidated in the study of physics and chemistry. Hence the understanding of biological systems and their development would seem to depend *inter alia* on an understanding of the physical processes involved, and must consequently benefit from a physics-motivated approach.

The Limitations of Linear Science

The classical approach to physics has been *reductionist*, attempting to reduce everything to its simplest constituents; this applies equally to the 'modern' physics of the very small and the very large — quantum mechanics and its successors, particle physics and field theories, and general relativity and cosmology — as to classical Newtonian physics. The phenomenal success of this approach is demonstrated by the remarkable accuracy of predictions in large- and small-scale physics, as well as by the triumph of modern-day technology.

The explanation of matter in terms of its building blocks, so effective in physics, has also paid handsome dividends in biology, with the greatest feat being the elucidation of the structure of DNA and the deciphering of the genetic code, which in principle contains all the information necessary to 'build an organism'; we have however already seen some of the limitations of this view. Hand-in-hand with the philosophical reductionism has gone a tendency to approach and model the world in terms of *linear* equations and effects; this was due partly to the unmanageable difficulty of solving nonlinear equations analytically, but also to the success of such a linear paradigm, probably exemplified most strongly and triumphantly in the reduction of the description of all electromagnetic phenomena to the linear Maxwell equations.

The tendency, indeed the active desire, to account for the world in terms of basic, fundamental units of reality enjoyed astounding and indisputable success, but it has become abundantly clear that this reductionism and linearization does not constitute the whole story, not even in principle. The manifest and increasing order and organization inherent in biological systems, in particular, has always defied the simple reductionism that has proved so successful in physics. Whereas the thermodynamic discoveries of nineteenth century physics portrayed a world in which disorder, or entropy, relentlessly increased and drove the universe inexorably towards a more random, less structured, less 'purposeful' state, evolutionary theory, the contemporary pinnacle of biological understanding, described a situation of continuously increasing complexity and information in living organisms, with an evolutionary 'arrow of time' that appeared to head in the opposite direction to the thermodynamic arrow. Equally mysterious was the embryological development of living organisms, where (once the preformationist 'homunculus' theory was discredited) researchers were faced with trying to explain the emergence of a structurally and functionally complex and ordered organism from a single cell.

In the face of the challenges presented by biology, linear physics must either restrict its ambit of investigation and validity to only limited realms of reality, or it must give way to physical insights more suited to approaching these questions. That physics, and science in general, is transcending the strictures of the narrow vision of a linear world is largely due to the enhanced computing power that has been available for the last few decades, enabling numerical investigations of the behaviour of nonlinear systems where analytical results are out of reach. But even more significant than the benefits of increased 'number-crunching' power has been the above-mentioned realization of the limitations of linear science, so that there has been a fundamental shift in scientific paradigms: "There are really three ultimate frontiers of physics: the very small, the very large and the very complex" [75, p.4].

The Promise of Nonlinear Science

We have come to the recognition that macroscopic effects may conceivably be compatible with, but not necessarily understood or accounted for by, their microscopic foundations, so that more global or holistic approaches are required in tandem with the analytic, reductionist modes of inquiry. Two seemingly counterintuitive and contrary situations are hence found to be possible [75]:

- Firstly, simple dynamical laws may give rise to complex behaviour, with exceedingly sensitive dependence on initial conditions — a small change in the initial situation can trigger an exponentially large divergence in the dynamical trajectories of the system, causing the system essentially to lose all predictability in spite of the complete determinism of the dynamics. Such complexity is not necessarily a function of a large number of degrees of freedom (we can understand why a system of, say, 10^{23} atoms would behave in a complicated way, but that three-body behaviour is also unpredictable is less expected, although recognised and shown already by Poincaré near the turn of the century). This sensitivity to initial conditions (combined for mathematical rigour with some other requirements, such as the density of periodic orbits and the topological transitivity of the attractor) is the basis of the fairly novel, but recently much-studies **chaos theory** (see also section 6.2.4).

- The second, converse situation is also essentially nonlinear in nature, and is that of **self-organization**, the 'unusual propensity of matter and energy to self-organize into coherent structures and patterns'. Vast numbers of, for example, molecules 'spontaneously' move into ordered behaviour, and behave in a cooperative way characterized by long-range correlations. This is the 'inverse' of chaos, in that for systems with many degrees of freedom one would intuitively expect complicated behaviour. In particular, it seems to fly in the face of the celebrated Second Law of Thermodynamics, mentioned above, which appears to require an increase in disorder, not in order, with time; that there is no conflict will be discussed later (section 3.1.2), but the tendency of some systems to self-organize certainly challenges the spirit and world view of the Second Law, which suggests a universe "running down amid spiralling entropy" [75, p.5].

Chaotic behaviour and self-organization, the decay of order in simple systems and the generation of order in complicated systems, appear to form two diametrically opposed poles of the new nonlinear view of the world, but in fact they are deeply connected. Although chaos

represents the breakdown of predictive science, there is an underlying mathematical order in chaos, expressed especially in the universality behaviour demonstrated by Feigenbaum, with scaling relations common to entire classes of systems, related to the renormalization group and to critical phenomena (see for example [318]). Conversely, systems which undergo self-organizing transitions, such as the Bénard convecting fluid cells and the Belousov-Zhabotinskii reaction, both to be discussed later, are also prone to undergoing chaotic transitions.

3.1.2 Prototype Self-Organizing Systems

As for other branches of science, the understanding of self-organization has been illuminated considerably by the consideration of a number of prototype systems, apparently simple systems whose behaviour displays certain intriguing characteristics. These systems do not nearly approach the complicated structure or intricacies of behaviour associated with biological systems, but it precisely for this reason that they have been invaluable in clarifying fundamental features of self-organization.

The Bénard Convection Cells

Introduction and Basic Features Possibly the simplest and best-understood example of a self-organizing system is a hydrodynamic one [10, 274]. Consider a thin, horizontal layer of a viscous fluid at rest between two 'infinite' horizontal plates (that is, their separation is much less than their lateral dimensions). The top plate is held at a constant and uniform temperature T_1, while the bottom plate is held at T_2. At first, at equilibrium, the temperature difference $\Delta T = T_2 - T_1$ is zero, and the fluid is at rest; essentially 'nothing interesting' happens, and any minor localized perturbations in temperature rapidly decay to the uniform state.

We can induce 'interesting' behaviour by heating the fluid from below, so as to generate a positive ΔT. The maintenance of the temperature gradient corresponds to an externally applied *constraint*, an energy input, preventing the attainment of thermal equilibrium. When the liquid is first heated, there is a continuous relatively small gradation in temperature throughout the liquid, and heat is transported by thermal conduction from the lower to the upper plate; there is essentially a linear relationship between the applied 'force', the temperature gradient, and the flux of heat through the system, in an essentially homogeneous and stable environment. As the heating is continued slowly, however, at a critical temperature gradient ΔT_c, the Bénard instability point, the fluid begins to perform a bulk movement; as a result of some small local temperature perturbation, internal convective motion occurs spontaneously. The fluid is organized into cells which display a spatial regularity, and roll, rectangular or hexagonal structures with a characteristic macroscopic size form, depending on conditions. At the molecular level the fluid particles move in unison in a cooperative fashion, so that the energy of the random thermal uncoordinated motion of the molecules has been, at least in part, replaced by the energy of *cooperative macroscopic ordered* motion.

The immediate cause of the organized behaviour is to be found in the amplification of density fluctuations: the heated fluid below is less dense, so that the force of gravity tends to pull down the cooler fluid elements above. A small downward displacement in the position of an upper fluid drop will bring the cool fluid into a warmer and hence less dense region, so that it will experience a downward Archimedes force which will tend to amplify the initial descent; similarly, heated

fluid that is displaced slightly upward will tend to rise even further. Thus a temperature gradient will tend to permit the formation of ascending and descending currents. But such currents do not occur for small ΔT since the destabilizing effects are counteracted by the stabilizing effect of of the viscosity of the fluid, which generates an internal friction opposing motion. As ΔT is increased, it is apparent that at some critical point the destabilizing temperature and hence density gradient gains the upper hand in the 'competition' with the stabilizing forces, and bulk convective motion appears. We have isolated the 'proximate' cause for the bulk motion, but the effect is ultimately to be ascribed to the nonequilibrium constraint of the applied temperature gradient.

Symmetry-Breaking and Long-Range Correlations Note that beyond the instability there has been a fundamental change in the symmetry of the system. Whereas in the uniform fluid each point in space is equivalent to every other point, after the appearance of the convection cells, adjacent rolls rotate in opposite directions, and points are distinguished by whether they are in a cell with clockwise or anticlockwise rotation; equivalent points are only found if one moves a distance of two rolls in the fluid. Hence we observe the phenomenon of translational *symmetry-breaking*, with the introduction of a characteristic spatial length determined by the boundary conditions and the size of the experimental apparatus, of the order of millimetres to centimetres. When one considers that this macroscopic order persists in spite of the magnitude of the characteristic scale of intermolecular forces (which have a range of a few Angstroms), and the fact that each molecule itself is undergoing random thermal motions, the remarkable fact of the existence of *long-range correlations*, generating the coherent motion of a huge number (of the order of 10^{20}) of particles, becomes apparent.

The Effect of Random Fluctuations There is an aspect of *non-uniqueness*, and concomitant 'choice', in the possible behaviours of the Bénard system. Beyond the critical temperature gradient ΔT_c, the fluid becomes structured into alternately left- and right-handed, or clockwise and anticlockwise, rolls. This is a perfectly reproducible situation, and once a direction of rotation is established in a particular cell, it will not change. However, for any (arbitrarily chosen) 'first' cell, the direction of rotation is unspecified, and could be in either direction; although once the rotation in one cell is chosen, all others are determined. The system has two equally likely behaviours, both completely compatible with the macroscopic equations of state (of for example hydrodynamics or thermodynamics), so that we are faced with the situation of *bistability* — two (or more, in general) stable states can coexist.

In any given Bénard experiment the rotation of the first cell is completely unpredictable and uncontrollable, although one of the two possibilities is guaranteed to occur; the direction of rotation is 'chosen' by the direction of random fluctuations, or small vibrations, that are inherent in every physical system at nonzero temperature. Hence statistical fluctuations are an inherent part of the self-organization; but once the fluctuation has occurred, it is amplified with probability one, that is, completely deterministically. We can thus speak of the *interplay between 'chance and necessity'* bringing order and coherence. Also, once the initial 'choice' has been made, the system configuration at all later times retains a 'memory' of the past event, the fluctuation, which took place at an earlier time in the system evolution and which played a critical role in all future behaviour — so the time development of the self-organizing system displays a *historical dimension*, so readily apparent in biological development and evolution.

Further Features of the Bénard Cell As ΔT is increased beyond ΔT_c, new self-organized phenomena appear, in a sequence depending on the fluid and experimental conditions. Initially the Bénard cells are maintained, but some of their characteristics are modified. With increasing temperature gradient, a new critical value is reached at which, locally, the velocity and temperature of the convection cells change periodically in time, with a constant frequency and in a predictable fashion. This régime prevails until a new threshold is reached, beyond which the velocity and temperature change become quasi-periodic.

Eventually, another well-defined critical temperature gradient $\Delta T_c'$ is attained beyond which the structure is 'lost', that is, it becomes fuzzy, and a régime characterized by erratic dependence of the fluid variables on time will emerge, giving *chaotic* or *turbulent* behaviour. The self-organization is thus not confined to the initial symmetry-breaking, but manifests itself in a succession of ordered behaviours appearing at discrete values of a parameter, in this case the temperature gradient ΔT. The Bénard experiment serves as a clear demonstration of several important features of self-organization which we will encounter later. (For introductory discussions of the Bénard experiment, which we have followed here, see for example [10, 141, 274, 277, 303].)

Other Prototypical Situations, in Chemistry and Biology

Thus far we have examined a self-organizing system which falls within the realm traditionally considered as physics, as the chemical nature of the fluid constituents remains unchanged during the convection phenomenon. To gain a deeper knowledge of self-organization, and to approach our ultimate goal of understanding in developmental biology, it is profitable to consider the classic examples in chemistry and biology — the Belousov-Zhabotinskii reaction, and the aggregation of the cellular slime mould *Dictyostelium discoideum.*

The Belousov-Zhabotinskii Reaction The much-heralded Belousov-Zhabotinskii (BZ) reaction displays so many of the characteristic features of self-organizing systems that we seek, and displays so clearly the usefulness of the kinetic, mathematical approach and techniques that will be introduced later, that it is discussed in some depth in appendix B. There its mechanism is described, and models that are able to account in detail for the observed behaviour, which includes temporal oscillations, concentric and spiral wave patterns, and more complex three-dimensional spatio-temporal structures, are outlined. Allied chemical reactions have also been shown to display stationary spatial structure formation.

Here again we have the spontaneous generation of structure and breaking of symmetry, in a nonequilibrium situation characterized by autocatalysis and an open system maintained by a mass flux of reagents through the system. In this case, temporal symmetry-breaking is especially pronounced, as fixed-period oscillations, circular or spiral wave patterns introduce a characteristic time scale into the system, which was previously invariant under translation in time. Such *spatial or temporal symmetry-breaking* is one of the fundamental characterizing features of self-organization. For further discussion of these chemical systems and their features and analysis, see appendix B, or the extensive introductions (with references) given for example in [10, 98, 245, 251, 276, 277, 303, 355].

The Cellular Slime Mould Biological development occurs, by definition, only in multicellular organisms; but much useful understanding of the processes involved, together with a detailed quantitative model, has been achieved in a class of organisms, the cellular slime moulds, that may exist both independently, as single-celled creatures, and in organized cell communities with spatial structure and differentiated cell functions. Best understood is the slime mould *Dictyostelium discoideum*, which generally maintains a unicellular existence, but undergoes aggregation behaviour under starvation conditions; a periodic chemical signal pulse serves to trigger a phase of motion inward towards aggregation centres, and thus stimulates self-organizing behaviour which has been modelled with fair accuracy. This (relatively) simple biological system, already referred to above in section 2.2.1, will be discussed in more depth below, in section 3.3; it suffices here to say that the conditions for self-organization are again satisfied, and many of the characteristics of self-organization described above for the Bénard experiment also feature here.

3.1.3 The Thermodynamic Feasibility of Self-Organization

We have noted the existence of self-organizing phenomena in diverse physical, chemical and biological situations, which aid in the establishment of a framework within which biological self-organization in development may be understood. Such a conceptual scheme must at least be *consistent* with physico-chemical laws, even if it may not necessarily be deduced *a priori* from them, for biological systems and organization must fall within the range of validity of physical theory. Here we come up against a difficulty, as mentioned above, for the generation of biological order and structure appears to be in direct opposition to a major legacy of nineteenth century physics, the Second Law of Thermodynamics.

The Second Law Prohibits Self-Organization? This (macroscopic) law, in its simplest form, proclaims the 'inexorable triumph of disorder'. More formally, the entropy in any isolated system has to increase with time; $dS \geq 0$. In consequence of the Boltzmann microscopic interpretation of entropy in terms of the number of complexions, or possible states available to a system, the equilibrium state of maximum entropy was historically interpreted as maximal 'disorder' or randomness, clearly in conflict with the coming into existence of structures of greater and more intricate structural and functional complexity as described both by Darwinian evolution and by development. The leeway provided by statistical mechanics, that allowed random processes to generate order by pure statistical fluctuations, near equilibrium and at sufficiently low temperatures for any organization not to be disrupted by thermal noise, was clearly insufficient, for the probability of formation of biological structures of any complexity at ordinary temperatures, beginning with the macroscopic number of molecules required to be assembled, is negligible. How then can order be created spontaneously?

The harmonious coexistence between thermodynamics and the concept of self-organization has been facilitated by the extension, in the last few decades, of classical thermodynamics to irreversible processes and nonequilibrium situations, notably by Prigogine, Glansdorff, Nicolis and co-workers in the 'Brussels group'. We will here only outline the chief concepts that pertain to this study, which established the thermodynamic feasibility of self-organization; for elementary introductions, refer to [277, 312], or for more detailed and comprehensive discussions, see for example [10, 303] or the original work well summarized in [116, 276]. Much of the discussion in this section 3.1.3 closely follows the account of Peacocke [303].

Thermodynamics and Dissipative Structures

An isolated, or closed, system is one that has no exchanges of matter or energy with the environment; for a non-isolated, or open, system, where such exchanges do occur, the entropy variation is the sum of two terms:

$$\frac{dS}{dt} = \frac{d_i S}{dt} + \frac{d_e S}{dt}, \tag{3.1}$$

where the entropy flux $d_e S$ is due to the exchanges, and the entropy production $d_i S$ is due to irreversible processes occurring within the system (reversible processes do not create entropy). The extension of the Second Law to this case is $d_i S \geq 0$, which corresponds to the normal statement for an isolated system, in which $d_e S = 0$.

If $d_i S$ is strictly positive, irreversible processes are continually occurring in the system, which we thus term **dissipative** — the evolutionary equations of the system are not invariant under time reversal. Common irreversible processes contributing to $d_i S$ are chemical reactions, heat conduction, diffusion, viscous dissipation, and relaxation phenomena in electrically and magnetically polarized systems. For each of these processes (indexed by α), two quantities may be defined: the generalized forces X_α (such as temperature or chemical potential gradients) which maintain the nonequilibrium situation, and the corresponding internal flows, or fluxes, J_α (such as bulk convective or diffusive motions), denoting essentially the rate of the process. It is remarkable that, at least for the range of phenomena amenable to a local description similar to that afforded by hydrodynamics or chemical kinetics, the entropy production $d_i S$ may be expressed as a bilinear form of the X_α and J_α:

$$\frac{d_i S}{dt} = \sum_\alpha J_\alpha X_\alpha. \tag{3.2}$$

Classical thermodynamics concerned itself first and foremost with *equilibrium processes*; those that are characterized by detailed balance, which is a manifestation of the *time reversibility* of the elementary processes occurring in the system. At equilibrium all generalized forces X_α are zero, and there is no macroscopic transport of the constituents, so the fluxes J_α are zero. Any small fluctuations, for example localized flows or concentration imbalances, can be shown to decay, so that random disturbances cannot bring about any ordering.

Once we look beyond the thermodynamics of equilibrium and reversible processes, we may however find support for macroscopic organizing behaviour. Nonequilibrium processes are associated with nonvanishing fluxes between the system and the environment; these may be transient, but can also be permanent if we impose and maintain appropriate *constraints*, for example the temperature gradient ΔT applied in the Bénard experiment. In consequence of the action of the constraint, detailed balance will not hold in the nonequilibrium situation, so that a regime of nonequilibrium becomes susceptible to change; localized small attempts to deviate from it are not necessarily obliterated by an instantaneously developed counteraction, but may rather be accepted and even amplified by the system, thus becoming sources of innovation and diversification.

As an indication of how this might come about, consider again equation (3.1). Whereas, as we have emphasized, the Second Law imposes $d_i S \geq 0$, there is no corresponding restriction on the sign of the entropy flux $d_e S$; it is thus conceivable that $d_e S$ can become sufficiently negative and exceed the magnitude of $d_i S$, so that the evolution of the system in (3.1) could

be characterized by $dS/dt < 0$ — then according to the traditional interpretation, disorder decreases or order increases in the course of the evolution. Hence the creation of order at least appears feasible in such open, nonequilibrium systems which are able to 'export excess entropy' to the environment.

Linear Nonequilibrium Thermodynamics Such *plausibility arguments* are, however, as yet insufficient to establish the possibility of self-organization. The initial extension of thermodynamics to the irreversible range was to the region near equilibrium, where the forces are still fairly weak; in this region the flows and rates of the processes may be expanded as *linear* functions of the forces, by $J_\alpha = \sum_\beta L_{\alpha\beta} X_\beta$, where the $L_{\alpha\beta}$ depend on the internal structure of the medium. It may be shown, using arguments based on the principle of microscopic reversibility and fluctuation theory, that the Onsager reciprocity relations, $L_{\alpha\beta} = L_{\beta\alpha}$, hold.

In this range, nonequilibrium steady states characterized by a lower entropy than the adjacent equilibrium steady state may exist, as hinted at above; but the steady states along the so-called 'thermodynamic branch', which are obtained as a smooth systematic deviation from equilibrium by the gradual change in some parameter or constraint, are still asymptotically stable, so that small perturbations decay. In this linear range *some* ordering is possible as a result of the constraints, or boundary conditions, on the system, but this 'order' is not really structural and is far from the organized intricacies of biology; so that even though entropy might decrease, the problem of creation of low entropy, complex systems is not yet solved by the linear nonequilibrium analysis. In the Bénard experiment, for example, the linear range corresponds to the regime of heat conduction in the temperature gradient range $0 < \Delta T < \Delta T_c$.

The Nonlinear Range and Instability For the proper description of biological processes we must move beyond a linear account, for biochemical processes involve *feedbacks* which introduce *significant nonlinearities*. The theoretical study of thermodynamic systems in the nonlinear range, far from equilibrium, has yielded a general evolution criterion [116], which prescribes permissible directions of change under nonlinear nonequilibrium conditions, provided that local equilibrium prevails. This criterion by itself does not guarantee the stability of any nonequilibrium state; so we consider fluctuations about some nonequilibrium reference state, and define a quantity called the *excess entropy production*, $\delta_X P$, which is given by the changes in the entropy production consequent only upon small but finite changes δX_α in the forces X_α from their steady state values. The positivity of this excess entropy production, that is $\delta_X P \geq 0$, guarantees the stability of the nonequilibrium state under consideration.

The above is only a sufficient condition for stability — its violation does not necessarily lead to disorder — but if it is not satisfied, then the system *may* be unstable, and fluctuations, instead of regressing to zero, may grow and the entire system may become unstable, leading to the establishment of a new structural order. There is thus the possibility that fluctuations could occur which will cause the system to break away from the thermodynamic branch, to rupture its continuity with the linear range, and so to pass to a new stable regime of a different kind that generally has a lower value of the internal entropy S_i and may well be more coherently ordered. In order to assess whether or not this actually occurs, we need firstly to incorporate the study of fluctuations into our more general thermodynamics, which will be touched on below, and secondly to make use of other methods of analysis, in particular kinetic approaches, which will

be considered in detail later in this chapter.

The study of nonequilibrium thermodynamics thus indicates that the distance from equilibrium and the nonlinearity may both be sources of order, capable of driving the system to an ordered configuration. These ordered configurations that emerge beyond an instability of the thermodynamic branch of nonlinear systems have been called **dissipative structures** [276], because they are created and maintained by the entropy-producing 'dissipative' processes occurring inside the system through which, being open, there is a constant flux of matter and energy. In the light of the above thermodynamic considerations, certain conditions for the generation of structure, or self-organization, may thus be deduced; they are that a process of self-organization can occur in a system if:

1. The system is *open* to the flux of matter and energy;
2. The system is not at equilibrium and preferably *far from equilibrium*; and
3. The system is *non-linear* in its force-flux relationships, that is there must be strong coupling between its processes.

These requirements are certainly satisfied by all biological systems, and so the following 'theorem' formulated by Nicolis (1974) becomes applicable, and serves to summarize the whole analysis:

> Consider a single phase system satisfying the above three prerequisites, whose entropy can be defined in terms of macroscopic quantities. Under these conditions, steady states belonging to a finite neighbourhood of the state of thermodynamic equilibrium are *asymptotically stable*. Beyond a *critical distance* from equilibrium they *may* become unstable. [272] (cited in [303, p.62])

Order through Fluctuations

The possibility of the formation of ordered, dissipative structures discussed above, with the final configuration being triggered by and depending on perturbations that drive the system away from the 'thermodynamic' steady state, has been publicized by Prigogine as 'order through fluctuations'. The role of *stochastic processes* in self-organization has been considered in some detail, especially by the Brussels school — see for example [276]; Haken [141] utilizes a slightly different approach.

For an ideal system in equilibrium, the distribution of fluctuating variables is a Poisson distribution:

$$\mathrm{Pr}_X = e^{-\bar{X}} \frac{(\bar{X})^X}{X!}, \tag{3.3}$$

where Pr_X is the probability of the fluctuating variable having the value X when the mean value is $\bar{X}$; this distribution prevails also for linear nonequilibrium situations. The Poisson distribution reflects the fact that only microscopic correlations, on the characteristic scales of the intermolecular forces, exist; hence the fluctuations in any parameter are negligibly small compared with the average, macroscopic values.

For systems sufficiently far from equilibrium, however, fluctuations may increase and depart from a Poissonian distribution to a sufficient extent that long-range spatial coherences, manifested in spatial correlation functions of finite amplitude and macroscopic correlation lengths,

may appear. In such a régime large-scale fluctuations over the range of macroscopic volumes comparable to the size of the system itself can occur, which may cause spatial or temporal symmetry-breaking and drive the system into a new macroscopic state, with new average values of its parameters.

Nicolis [273] has examined more fully the relation between the dynamics of fluctuations in systems far from equilibrium and the macroscopic behaviour, in relation to internal fluctuations generated spontaneously by the system itself; his analysis summarizes the situation well. The behaviour of the systems under study evokes a question: Self-organizing systems

> ...can exhibit coherent behaviour which manifests itself in the form of regular spatial patterns or of rhythmic phenomena emerging abruptly in a hitherto homogeneous and stationary system. Both phenomena imply sharp and reproducible correlations between distant parts of the system. How can such coherence arise in a dilute solution of molecules behaving almost like hard spheres, and hence incapable of recognizing each other over more than a few Angstroms?

He answers this question himself by showing that it is *deviations* from a Poissonian distribution that are responsible for the appearance of correlations and structure:

> One can show that a spatial *correlation function* between distant parts of the system will emerge whose amplitude is directly proportional to the non-Poissonian part of the fluctuations. In summary, deviations from the Poissonian are the triggers that enable the system to deviate from the macroscopic regime and hence to choose between many available solutions in the presence of bifurcation phenomena. Normally, in the limit of a large volume, one may neglect them. But in some cases, the deviation from the Poisson behaviour becomes comparable to the average value itself ...Note that diffusion, which is always present, tends to re-establish the Poissonian in a scale of the order of the mean free path ...However, the competition with chemical kinetics may give rise to a deviation from the Poissonian in a macroscopically large sub-volume of the system. Subsequently, this large fluctuation may propagate throughout the system and drive it to a new macroscopic state. Molecular dynamics studies, whereby the chemical reactions and the thermal motion of the molecules are simulated on a computer, confirms the existence of different laws governing fluctuations of different scales. [273] (cited in [303, pp.147–148])

Thus we have the constant interplay between determinism and stochasticity, between chance and necessity, which plays such a fundamental role in self-organization.

In the rest of this work, however, we will restrict ourselves to the *deterministic* dynamical behaviour of the system, and consider fluctuations as givens, of unspecified small magnitude, which may serve to expose and exploit the instability of stationary states.

Usefulness of the Thermodynamic Approach

Originally it was thought that the 'dissipative structures' whose existence is postulated above, were a property purely of the macroscopic constraints which drive a system far from equilibrium, so that they arose from the macroscopic principles of thermodynamics, chemical kinetics,

hydrodynamics, or the boundary conditions imposed on the system. Recent work, using computer simulations of dynamical algorithms in molecular dynamics (for example modelling the Newtonian dynamics of fluid particles in the Bénard problem) has however shown that the self-organization is a property of the *unstable dynamical laws* as such, without any macroscopic assumptions (see [307] and citations therein). Hence the macroscopic framework of thermodynamics is not *indispensable* in understanding the creation of dissipative structure, but is rather a crude description obtained from the averaging of microscopic phenomena. Furthermore, no biological organisms, or parts thereof, have been examined experimentally in sufficient depth with respect to their thermodynamic parameters to allow any detailed test of the thermodynamic ideas described above. So in what sense are the above thermodynamic considerations useful at all?

The thermodynamics of dissipative structures is clearly validly applied to the problems of biological organization, as biological systems are by hypothesis not at equilibrium (which is equivalent to the death of an organism); they are highly internally and externally coupled and so are nonlinear; and they are systems open to the steady flow of energy in the biosphere. The analysis of thermodynamics far from equilibrium has provided a criterion which is necessary for instability, and so delineates the parameter ranges within which the generation of non-trivial structure may be sought; however, it is not a *sufficient* criterion, so that supplementary information to confirm instability is needed. This can only come from a direct analysis of the rate equations for the processes occurring in the biological system, or in a model that seeks to represent some aspect of it; such a dynamical systems approach, based on the actual or hypothesized kinetics of the system, indeed forms the major technique utilized in this thesis to demonstrate pattern formation (see section 3.2, and appendix A).

Thus thermodynamics can never work in isolation from other approaches, such as those based on the theory of fluctuations, of stability, of stochastic processes and of nonlinear differential equations; however, it has its own insights which serve to link reflection on biological systems with the whole corpus of physico-chemical theory [303]. In particular, the structural order we seek to account for ultimately derives from the existence of constraints, and the macroscopic and phenomenological approach of thermodynamics is uniquely fitted to handle such factors. Thus thermodynamic analyses can, for example, help in restricting the rate laws that might be incorporated into mathematical analyses and models, by eliminating some putative models of biological situations as being incompatible with macroscopic physical laws, and permitting others, whilst not actually determining the choice between different feasible models.

But the primary and overriding gain of the thermodynamic approach is undoubtedly in the ability of thermodynamics to provide "an architectonic skeletal framework" for the understanding of self-organization, which "limits but does not in detail prescribe" [303, p.71]; thermodynamics may be well characterized supremely as the 'science of the possible'. In particular, irreversible thermodynamics has broadened our conceptual understanding by providing the concepts of 'order through fluctuations', in situations where nonequilibrium constraints are decisive, and of the possibility of spatial and/or temporal dissipative structures in open systems, together with the conditions governing the transitions between them. One may build on this framework of fundamental thermodynamic concepts by making use of other available mathematical and physical tools, such as dynamical systems theory, kinetics, and fluctuation theory; of course, for biological applications the most important is precise experimental information and new knowledge of the modes of control and regulation at all levels (in this discussion, we have

closely followed Peacocke [303, section 2.9]).

Ultimately, the thermodynamic concepts have deepened and broadened our perspectives on biological systems and whole organisms, and consequently their development. They have cleared the path of physico-chemical objections, and have hence been a stimulus for most of the detailed work on self-organization that is the foundation of the developmental models which will be described in the later sections and chapters.

3.1.4 Synergetics: the Analogy with Phase Transitions

The thermodynamic discussion above has introduced the phenomenon of the amplification of small fluctuations, which drive the system to a new ordered phase, characterized by different, lower symmetry compared to the initial more homogeneous situation. This behaviour is immediately reminiscent of an analogous situation in second-order *phase transitions* (see [274]). Consider for example the magnetization or demagnetization of a ferromagnetic material: there is a critical temperature, in this case, above which the material is isotropic, in the sense that there is no preferred direction, but below which a magnetization emerges; the rotational symmetry characterizing the unmagnetized state is broken, and the material becomes anisotropic. Similarly, the freezing of (isotropic) liquid water to an ice crystal lattice, with the solid state characterized by rigid order and a well-defined spatial scale, and the concomitant loss of translational symmetry, is another phase transition. For first order phase transitions involving nucleation and metastability, a similar destabilizing role to the infinitesimal fluctuations of second order transitions, is played by finite fluctuations.

Much of the initial work on self-organization proceeded on the basis of this striking analogy between phase transitions and nonequilibrium transitions at a critical, or bifurcation, point; for in both classes of systems beyond the critical points, the fluctuations increase, departing from a Poisson distribution, and finally drive the system to a new macroscopic state. The major example of this approach has been the school of thought of '**Synergetics**' [140, 141, 142], led by Haken in Stuttgart, which attempted to play a similar unifying role in the study of self-organization to that aspired to by the proponents of dissipative structures in Brussels.

This school has sought to characterize and account for the deep analogies between superficially completely different systems, which all display the cooperation of individual parts of the system to produce macroscopic spatial, temporal or functional structures, in a manner which appears well-regulated or even purposeful. In its search for general mechanisms or principles that govern the cooperation of subsystems, irrespective of the nature of these subsystems, synergetics has appealed in particular to the concept of *order parameters* arising out of the theory of phase transitions: the study of the dynamical behaviour of the system, which may require a description in terms of very many or even a continuous range of variables, may essentially be reduced to the study of a small number of variables, the order parameters, such that the behaviour of all the other *slaved* variables is determined fully by the order parameters. This concept is discussed in some more depth in appendix A.3, in terms of its relation to bifurcation theory.

Limitations of this Approach The mathematical correspondence between the descriptions of phase transitions and self-organizing symmetry-breaking phenomena has provided a useful

basis for dealing conceptually and quantitatively with self-organization in terms of previously well-understood ideas; especially, as already noted, in the field of synergetics, where 'generalized Ginzburg-Landau equations for nonequilibrium phase transitions' have been developed and applied to self-organization [141]. But self-organization, and especially the generation of physical and biological complexity, is considerably more than mere phase transition, and we need to consider the intricate interplay between interacting, competing and adaptive entities; the analogy can only be taken up to a certain point.

There is another fundamental distinction, related to the fact that phase transitions are *equilibrium* phenomena, occurring by the minimization of free energy, and with a characteristic scale length (for example the lattice constant of a crystal) which is microscopic and comparable to the range of the interactions. In contrast, for self-organization behaviour, the characteristic scales of temporal and spatial patterns are macroscopic, and the systems are far from equilibrium, as already discussed. Thus the analogy with phase transitions, albeit useful, should not be exploited too far [274].

3.1.5 Complexity

We have noted above the characterization of self-organization as the 'spontaneous generation of complexity'. Intuitively, this corresponds to our understanding of 'complexity' — when a system loses symmetries and gains structure, it becomes more ordered, more intricate, more complicated, more 'complex'. This term has, in fact, taken on more than one general scientific meaning, and it is well for us to examine the concept of biological complexity in some more detail in order to improve our conceptual understanding of biological organization.

A Recent Scientific Fashion

In some scientific circles, the last few years have seen an explosion of interest in **'complexity'**, comparable to the interest in chaos theory in the past decade and, in fact, to dissipative structures, synergetics and catastrophe theory (which we discuss in some more depth in chapter 6) before then; the most enthusiastic proponents of all these schools of thought have effectively proclaimed them as 'theories of everything', capable of accounting for entire hitherto mysterious categories of phenomena. The popularity of complexity theory is evidenced by the recent publication of two books [204, 360], and by the rise to prominence of the Santa Fe Institute. The considerable public interest is also demonstrated by the appearance of popular articles such as the *New Scientist* supplements of 6 and 13 February 1993, and the article *Life, the Universe and Everything* (!) (*Time*, 22 February 1993). It is, however, not abundantly clear whether the concept is significantly more than just an advance on or reformulation of the 'old' concept of self-organization, possibly expressed in the framework of dissipative structures or of synergetics; in this section we shall consider some approaches to the study of complexity, in an attempt to gain some comprehension of this set of concepts (and its potential relation to biology).

The Concerns of Complexity Theory The domain of complexity 'theory' is in the difficult, and hence interesting, area between the systems that are fairly predictable and consequently essentially unexciting — those that have steady states, periodic attractors and so on; this en-

compasses much of classical physics — and those that are so complicated that they are really just a huge mess, with little ordered behaviour, and which can at best, when one is fortunate, be described statistically. Complexity concerns itself with the middle ground, with "those that hover in between, structured but unpredictable, a flux of almost-patterns ...the mysterious 'edge of chaos' " [334].

This distinction between different ranges of complexity in nature was first made by Weaver (1948) (see [102, 303]), who identified three distinct categories: *organized simplicity*, where problems could be reduced to the study of a small number of significant factors; *disorganized complexity*, with systems comprising enormous numbers of variables behaving largely randomly — both of these cases are eminently quantifiable, the former by analytic methods, the latter by the calculation of average properties using statistical techniques — and a large intermediate domain, that of (possibly) *organized complexity*, where the number of significant variables was much greater than two and still much less than, say, a million. Here we deal with self-organization arising from the nonlinear dynamics of systems with many (reasonably similar) interacting components, in such a way that various large-scale phenomena emerge naturally and predictably.

Weaver noted that this field of study was largely untouched, and urged that the important problems of organization, of the interrelation of a sizeable number of factors integrated into an organic whole, should be tackled. This is now being done with some degree of success and considerable enthusiasm, with the investigative techniques depending on the great recent strides made in computing power; so that the contemporary study of complex systems involves predominantly computer simulation of large interactive models, in contrast to the study of self-organization, which has depended on the analysis of simple paradigmatic cases and model equations such as reaction-diffusion systems (which will be introduced and studied in depth later).

Anti-Chaos A popular theme in complexity theory is that of '*anti-chaos*', promoted especially by Kauffman (see for example [179]), who has worked also in the field of developmental biology [180]. He has, for instance, idealized gene control systems by modelling them as random Boolean networks, where the gene is considered a simple on/off switch, and the genome is treated by analogy with a complex parallel-processing computer or network. He has simulated the behaviour of these networks, and concludes that structure emerges out of adaptation to the environment, which serves to *narrow* the range of possible internal states by 'freezing' large components of the system variables and restricting others to predominantly stable attractors in a feedback control mechanism. The feasibility of this mechanism of anti-chaos has been brought into question, on the grounds that emergent structure is not based on the narrowing of possibilities, but is rather a matter of coherent large-scale function which 'rides on and is irreducibly driven by' an incredibly intricate internal structure, but one that is 'transparent' to the large-scale function [334].

Holism in Complexity More fundamental to complexity than the detailed mechanism proposed above is the *philosophical aspect*, exemplified by the emphasis on the need for a *holistic viewpoint*: many of the phenomena observed in the systems under consideration, such as self-organization, increasing complication and sophistication, and unexpectedly long periods of qui-

escence interspersed with sudden bursts of wild activity, are baffling to a reductionist perspective which seeks to explain the behaviour of the system in terms of the properties of its parts. "The underlying thesis of complexity theory is that traditional reductionism — understanding systems by breaking them down into components and analysing the interactions between them — cannot provide an adequate understanding of such systems. It is the old problem of 'emergent phenomena', of wholes that somehow transcend their parts — indeed of wholes that are to a great extent *immune* to the detailed structure of their parts" [334]. This concept is quite basic — we need to progress beyond simple reductionism, the old 'linear' view of the world, to see the whole in its interactions (it is interesting to note that such a view of wholeness, of large-scale and long-time correlations, has also appeared in recent reappraisals of the foundations of quantum mechanics, exemplified by the experimental verification of the violation of Bell's inequality).

Such a holistic viewpoint, with the emphasis on considering the 'big picture' of global organization, of mechanisms, controlling principles and paradigms, is especially fundamental to our present study, if the consideration of development is to entail any more than a mere cataloguing of gene expression patterns and protein concentrations in the space-time development of an organism. The discussion given in chapter 2 has clearly indicated that the proper understanding of development requires an appraisal of how the overall control occurs, with such exquisite sensitivity and creation of fine detail and yet stability and regulatory or error-correcting ability; we need the *global* picture, the pursuit of which may well be facilitated by a complexity-driven viewpoint, for, as Stewart points out, "Despite — maybe because of — an immense reductionist attack on the molecular structure and function of DNA, we really know very little indeed about biological development: about form rather than chemistry" [334]. Thus he considers that the most significant contribution of complexity theory may be "in how it shifts the scientific goalposts away from ever-more-detailed analysis of fine internal structure towards a more global explanation of forms, features and functions" [334].

On the other hand, the tendency toward too much of a global viewpoint may also be dangerous, as the balance between the detailed study of underlying mechanisms and processes, and the development of unifying principles — that is, between the complementary analytic and synthetic approaches in science [151] — must be maintained. Especially in development, there has sometimes been a tendency for global explanations to be proposed 'in principle', with little cognisance being taken of the experimental facts of embryology, as we will discuss with examples in the course of the later chapters; such a critique may for instance apply to generalized field approaches (section 5.3.4) and catastrophe theory (section 6.1). In development there is a dichotomy between the microscopic and macroscopic features, which interact intimately, each influencing the other constantly and in a complicated way. Thus the holism emphasized by complexity theorists is laudable, with its message of "...it is time science relearned to look outwards as well as inwards, to think about meaning as well as counting information, and to appreciate nature's semantics as well as its syntax" [334], but should not be advocated excessively and uncritically.

Characteristics of Complexity

For any discussion of complexity to be useful and meaningful, it is important to have a clear understanding, and preferably a rigorous definition, of the concept; otherwise complexity-related questions, whether related to more physical aspects, such as issues in statistical physics or the

theory of computation, or concerned with topics in biological organization, may not be posed rigorously enough to be amenable to proof or refutation [28]. As already stated, however, no generally accepted definition of complexity exists, in spite of the recent surge of interest, and in fact there are divergent strands of thought concerning the themes which are to be considered central to complexity. We will consider two of these, focussing first on a more physics-directed approach concerning the evaluation of the complexity of states, and then on a systems-motivated analysis involving hierarchical structuring.

Bennett [28] has discussed various alternative definitions and features of systems that we would intuitively call complex from a physical and conceptual perspective, noting that natural irreversible processes appear to have a propensity for the spontaneous generation of complexity, or self-organization; we here closely follow his discussion. He points out firstly that, as we have already seen, the origin of complexity may be considered from various points of view — one can, for instance, be ambitious and study the origin of complexity on a philosophical or epistemological, or indeed a cosmological basis, or one may be more modest and restrict oneself to the analysis of the creation and destruction of complexity in well-chosen standard paradigmatic cases of models of many-body systems. These models may be discrete approaches of (possibly stochastic) cellular automata (see section 6.2), or continuous formulations through partial differential equations, such as those of hydrodynamics governing, for example, the Bénard system, or reaction-diffusion equations which will be considered in more depth later (see section 3.2 and appendix A). An obvious alternative is simply to attempt to elucidate the pertinent features of generally accepted 'complex' systems. Such analyses of putative 'complex' systems have revealed a range of proposed characterizations of complexity, which Bennett proceeds to list and consider. These may be divided into generally thermodynamic and predominantly computational features.

Thermodynamic Features An initially plausible candidate for complexity, namely *life-like properties*, is ruled out immediately, for such properties are firstly very hard to define rigorously — there is notable ambiguity about the 'living' status of viruses — and also too dependent on function; we would agree that a dead body is still complex, although functionally inert. Thermodynamic properties are also unsatisfactory: *thermodynamic depth*, the amount of entropy produced during a system's actual evolution, has been proposed, but it is can be too system-dependent, as much dissipation can occur *en route* to a trivial system, or comparatively little in arriving at a non-trivial state. Similarly, *thermodynamic potentials* themselves, such as free energy and entropy, although useful, are not directly related to our intuitive notions of complexity: a bottle of sterile nutrient solution, for example, has a higher free energy, but lower subjective complexity, than the bacterial culture which would result from the addition of a single bacterium to the solution. Thus the unlikelihood of the spontaneous generation of complexity is not a thermodynamic requirement, but rather the consequence of a putative 'slow growth' law that it seems reasonable to require complexity to obey: in our experience complexity tends not to increase quickly, except with very low probability.

Information-Theoretic and Computational Properties Characterizations of complexity based on information-theoretic or computational properties should also be considered carefully before being accepted. We have for instance found *long-range order*, the existence of statistical correlations between arbitrarily remote parts of a body, to be a property of self-organizing

or complex objects, but it is also found in intuitively simple objects such as perfect crystals. Similarly, *long-range mutual information*, also known as remote non-additive entropy, which measures the amount, rather than the range, of long-range correlations, has been proposed but is unsatisfactory as it may be produced very quickly, and thus does not satisfy the slow-growth law. *Fractal structures* and *chaotic dynamics* are also not necessarily related to complexity, in spite of the striking and often complex appearance of self-similar structures, and the fact that some intuitively complex entities are self-similar or at least hierarchical in structure or function; for such hierarchy is not apparent in all complex structures, and some self-similar structures are too rapidly computable, for example by the iteration of deterministic cellular automaton rules, to be termed complex in terms of the slow growth law.

With the above objective properties of the system itself found wanting, one may turn to the computations required to generate the system. A popular concept of complexity is that of *algorithmic information*, also called algorithmic entropy or Solomonoff-Kolmogorov-Chaitin Complexity [54], which measures the size (in bits) of the most concise universal computer program required to generate the object in question. This is closely related to statistically defined entropy, but for this reason it must be regarded as corresponding more nearly to randomness rather than complexity; using this definition, we would find that an (intuitively complex) literary text or genome is intermediate in algorithmic entropy between a totally random sequence and a perfectly ordered one [274]. Other computational approaches, such as *computational universality*, the ability of a system to be programmed through its initial conditions to simulate any digital computation, and *computational time/space complexity*, the difficulty of computing a given function, are too closely related to functions and functionality to be useful as a measure of the complexity of states.

Complexity as Logical Depth In the light of his criticisms of the other characterizations of complexity he discusses, Bennett proposes as his preferred characterization of complexity a concept related to *logical depth*, indicated by the execution time of a near-incompressible universal computer program to generate the object in question. Logically deep objects satisfy the putative slow-growth law by construction; they contain internal evidence of having been the result of a long computation or slow-to-simulate dynamical process, and could not plausibly have originated otherwise. For arbitrarily large times t, *a complex system is thus one which contains structures which could not plausibly have been generated in time much less than* t. In particular, biological systems are complex, by this criterion, in the sense that it has taken evolutionary time scales to produce them. Such a characterization of complexity seems reasonable, as it necessarily takes into account the irreducibility of complex systems and the quantity of information inherent in them.

This has been a very compact summary — for more details, see Bennett [28] and references contained therein — of a highly physics-motivated approach, which may not seem very relevant to biological development. It has however been included as it exemplifies one of the two very different contemporary approaches to the study of complexity.

Complexity as Hierarchical Organization in Systems

An alternative approach to the study of complexity, especially in biological systems, is one that takes into account the complicated interactions between subunits of a complex system; this approach is more specifically directed at living systems, although in principle it could also refer to any physical system and its dynamics. The explicit consideration of parts and their relationships in a hierarchical organization comprises the focus of *systems theory*, which may be described as being about 'dealing with complexity' [102].

The field of 'general systems theory' is concerned with the formulation and deduction of principles which are valid for 'systems' in general, based on the observation that one frequently observes structural correspondences or logical homologies between systems whose entities are of a wholly different nature. It aspires, very ambitiously, to be a new 'super-structure' for science, at least in the original motivation by Bertalanffy (1950) (see [303]); but it is not clear whether it has delivered any significantly new conceptual tools, putting it into the same category as other putative 'theories of everything'. Nevertheless, the holistic emphasis on systems may aid in the construction of a framework within which invariant aspects of models of physical or biological phenomena, and isomorphisms between systems, may be considered.

Hierarchies and Emergent Properties The fundamental feature pertinent to complex organization of systems is that of *hierarchical structuring* and associated *emergence*. Simon in his seminal paper on 'The architecture of complexity' (1962) defined a hierarchical system, considered basic to complexity, as "a system composed of inter-related sub-systems, each of the latter being, in turn, hierarchical in structure until we reach sone lowest level elementary subsystem" (the choice of which was regarded as arbitrary) (cited in [303, p.249]). We thus have a hierarchy of structural levels, each layer being built of subunits in the next lower layer, but embodying features that may not be meaningfully described or accounted for purely in terms of lower level concepts; the interactions of the subunits result in emergence, the introduction of *novel properties* that were not present at the simpler levels of organization.

Thus we meet again the breakdown of purely reductionist explanation, and the affirmation of features of higher-level systems that require for their description and articulation their own distinctive, non-epistemologically reducible concepts and language; in particular, we may emphasize the autonomy and non-reducibility of biology, the study of life, to purely physicochemical explanations and concepts. More immediately relevant to our present concerns, this means that biological development may not be understood in terms only of the reductionist insights of molecular biology and genetics; but equally not in terms only of overall dynamical models, for example. The general principle bears repetition: 'The whole is greater than the sum of its parts' (see Peacocke [303], while Ellis [94, ch.4] gives a more elementary introduction).

The interaction of a large number of parts or degrees of freedom constitutes complication; the hierarchical ordering introduces the organization that permits meaningful functionality in the system. A feature of natural hierarchies is that they are 'nearly decomposable' — that is, the interactions among sub-systems are relatively weak compared with the interactions *within* the sub-systems. We have a modular organization, of relatively independent, viable parts that are constrained to interact to generate functional, collective behaviour. Such modularity and hierarchical structure is fundamental to the creation of complex structures, especially in the light

of perturbations during the development; it can be shown (Simon, 1962) that the time required for the creation of a complex form from simple elements depends critically on the number and distribution of potential intermediate, stable forms, in particular if there exists a hierarchy of potentially stable 'sub-assemblies' (see [303, section 6.4]); this result is especially important to the conceptual understanding of the processes of both evolution and development.

In such complex, hierarchical systems, functionality is delegated to subunits whose structures are specialized to their functional roles. The coordination of the overall system then depends on control mechanisms to direct and constrain the behaviour of the subunits; hence *information flows* are essential to the organization in any complex system. For example, the genetic 'information' encoded by the DNA, together with structural, maternal, mechanical and other epigenetic factors, constitute the information flows that direct and maintain stable development. Such information (and concomitant energy) flows necessitate *nonequilibrium conditions* and spatial inhomogeneity, for the requisite flows to be possible; these we have already seen to occur in self-organizing and complex systems. The processes we are concerned with here, in order to embody useful information, will inevitably be *nonlinear*, as we have already discussed; and lastly, *feedback controls* are essential for the maintenance of stability in the complex system, to take corrective action to counter any imbalances, especially as complex systems are invariably open to the innovations, but also the perturbations, provided by the environment.

Other Approaches: Cybernetics and Network Thermodynamics A unifying description of the communication and information flows, and control systems, in complex systems, which relates to many of the issues alluded to here, is provided by the study of *cybernetics*. In addition to the general field of systems theory, a discipline which attempts to account for properties of arbitrary, general systems by formulating overarching concepts and principles (see for example [102]), a more restricted attempt to account for the complexity especially of biological systems has been made in *network thermodynamics*. This approach seeks to represent the interactions and hierarchies in biology, together with their spatial organizations and connections, from the viewpoint of power flow, and of energy dissipation and storage; it seeks to serve "as a bridge between classical thermodynamics and the general dynamic theory of modern physics, and [allow] the introduction of thermodynamic concepts into the systems approach devoted to the fundamental problems of biological organization" (Katchalsky (1972); quoted in [303, p.108]). The impact or usefulness of this approach in providing new concepts for the understanding of biological organization or complexity, especially in relation to issues of development, is however in doubt [303]. For further detailed discussions of the applications of systems theory and hierarchical organization in general, see for example [102]; for an extensive and useful examination of biological complexity in particular from this point of view, see [303] and references therein.

Complexity — A Well-Defined and Useful Concept?

We have considered complexity most recently from the viewpoint of hierarchical structure and constrained interactions between subunits at different levels in a complex system. This approach, directed specifically at living or purposeful systems, superficially appears quite far removed from the more physics-motivated description presented in the previous section, which attempts to depict the complexity of individual states. Nevertheless, underlying these two poles in the characterization of complexity is a fundamental unity in the nature of the phenomena being described

— after all, similar features, of openness to the environment, nonlinearity, and high information content, do underlie both descriptions, and the discussions above have been presented more to highlight the differences than the similarities — but it is clear that the concept of complexity is not a straightforward one. We are thus warned not to expect the study of self-organization and the development of structure, pattern and functionality, that is, complexity, in biological systems — which must constitute the apogee of complexity — to be anything but highly non-trivial and imperspicuous, especially to our current state of knowledge.

With this cautionary note in mind, we may proceed to investigate what *may* be understood about development, with the aid of models and concepts to deal with self-organization. We continue firstly with the development of some mathematical tools that have demonstrated their worth in the study of dynamical aspects of self-organization.

3.2 The Dynamical Study of Self-Organization

3.2.1 Dynamical Systems: the Language of Self-Organization

In our consideration of the feasibility of self-organization, we have found it useful and necessary to seek a thermodynamic justification for the spontaneous creation, rather than destruction and decay, of order; we found such support for self-organization in the concepts of irreversible thermodynamics far from equilibrium. In the process, we were led to introduce the ideas of equilibrium, stability and nonlinearity, which all play pivotal roles in the establishment of 'dissipative structures'. As we have already noted, however, the thermodynamic interpretations of physico-chemical processes and systems have both the strength and weakness, that they are fundamentally *phenomenological*, concentrating on the macroscopic properties of ensembles of particles, such as the molecules in some system under consideration, rather than on the microscopic details of molecular transformations and organization. Thus thermodynamics is at best able to indicate *potentialities* for evolutionary change in a system, but not prescribe the details of such change; it may constrain and permit, but not require, the direction of processes leading to self-organization.

The appropriate framework for the detailed study of self-organization is the mathematical language of *dynamical systems*. In the formulation of the time evolution of a system in the formalism, for example, of differential equations, we are able to consider, in principle, all system variables of interest (even though, as we shall find, the analysis and even numerical solution in general rapidly becomes intractable beyond a very small number of variables), and then make full use of the powerful tools of mathematical analysis to obtain the qualitative and quantitative behaviour of the system.

General Mathematical Formulation Thus, in general, the instantaneous state of a system may be described in terms of the dynamical variables $\{X_i\}$, which typically vary in time and space. For example, in a hydrodynamic system the X_i might refer to local flow velocities, temperatures, or pressures; in a chemical system the concentrations of reacting chemicals, or possibly local electric polarizations or fields, would be of interest, whereas we would focus on forces, stresses and strains in a continuum mechanical situation. The time evolution, possibly allied with spatial variations, of the system would then be modelled by a system of differential

equations, which can often be cast in the following general form:

$$\frac{dX_i}{dt} = F_i(X_1, \ldots, X_n; \lambda_1, \ldots, \lambda_m), \quad i = 1, \ldots, n. \tag{3.4}$$

Here the F_i denote the rate laws for the time rate of change of the state variables X_i (and will, in general, also include spatial differential operators), and the λ_i are the control parameters present in the problem, constants in any given dynamical situation but able to be modified by the external world [274].

A characteristic and crucial feature of the above formulation is that for the vast majority of systems encountered in nature, and definitely for any systems with the potential for self-organization, the F_i are *nonlinear* functions of the X_i; as we shall see below, in chemical reactions or biology, for example, this is related to the ability of certain molecular species or enzymes to perform autocatalytic and other regulatory functions. Linear equations are in some sense 'not very interesting', as they can be solved exactly and uniquely in general, for example by the application of infinite series methods; whereas a typical feature of nonlinear systems is multiplicity of solutions, giving rise to the 'choice' between various possible modes of behaviour a self-organizing system has to make.

Discrete Dynamical Systems It should be noted that a continuous, differential equation formulation of the evolutionary behaviour of a dynamical system is not always the most appropriate; indeed, frequently it is arrived at by the continuous approximation or smoothing of a fundamentally discrete system in order to have available the powerful tools of analysis. An alternative formulation which has recently become more popular is that of discrete dynamical systems, or cellular automata, which can frequently capture different or more realistic aspects of the solution behaviour, and can be implemented and studied numerically directly and easily (whereas the numerical solution of differential equations involves, indeed, a discretization). The self-replicating and self-organizing properties latent in some cellular automata were initially popularized through John Conway's "Game of Life" (see [106, ch.20–22]), and have been the subject of some recent intensive study, as will be discussed further in section 6.2.

Qualitative Solution Properties Once a continuous, differential equation or dynamical system formulation of the situation under study has been performed, we may use the techniques of dynamical systems theory to follow the evolution of the system. Possibly the most valuable perspective on such evolution is obtained by embedding it in *phase space*, that is by representing the instantaneous state of the system as a point in a space spanned by the state variables (the position and velocity or momentum of every particle in the system), and letting the succession of such states define a curve, the phase space *trajectory* (see for example [87, 277]).

We have already had cause to refer to dissipative systems, those for which the evolution equations are not invariant under time reversal; for such systems, for large times transient behaviours of the system die out, so that the trajectory tends to an object representative of the régime of organization of the dissipative dynamical system; this régime we call an *attractor*. It may be an equilibrium solution or *fixed point*, corresponding to a steady, homogeneous state; it may be a periodic or multiperiodic orbit, or *limit cycle solution*, in which case a time ordering has been introduced into the system; or it may be more complicated, with the most complex

and challenging known attracting object being a *strange* or *chaotic attractor*, characterized by sensitive dependence on initial conditions and exponential divergence of trajectories, so that chaotic behaviour provides an 'archetype' of natural phenomena characterized by limited predictability [274]. The goal of the study of self-organization in any system is then the search for new attractors arising in the system as it is driven away from equilibrium.

Chemical Kinetics A profitable domain for the formulation and analysis of models of self-organization, with as we shall see particular applicability to biology, is in the kinetics of chemical change. The state of the chemical or biochemical system of interest is described by the chemical concentrations, say $u_1, \ldots, u_n$, of the n component chemical species in the system which have been isolated by experiment or theoretical simplification to be important to the dynamical behaviour of the system; the substances interact with each other, and the direction of the change is governed by the *rate laws*, which comprise the dynamical laws of motion for the system. Such a chemical formulation links up naturally with thermodynamic concepts, which will allow us below to compare the predictions of these two approaches. We will for the present be largely concerned with models of self-organization that have their physical justification in the interactions of chemicals, but it should be noted, as pointed out above, that the dynamical variables need not necessarily refer to chemical concentrations, and we will in chapter 5 consider self-organizing behaviour displayed by mechanical and other systems.

3.2.2 Reaction-Diffusion Equations as a Paradigm for Self-Organization

The standard formulation of chemical rate laws considers purely the time evolution of the concentrations of the chemicals under study; spatial dependencies are ignored. This is generally justified by the phenomenon of *diffusion*: spatial variations in concentrations tend to be smoothed out, since the random thermal behaviour of molecules in all directions, when occurring in a spatially heterogeneous environment, results in a net migration of molecules from a region of high to low concentration of the chemical species. Macroscopically, this corresponds to motion down a chemical potential gradient, and is modelled as diffusion down a concentration gradient (see section 4.2.1). The mathematical formulation takes diffusion into account by modelling the flux of the chemical as proportional to the spatial change, or gradient, of the concentration, ∇u, with the proportionality being denoted by the diffusivity, D_u, which in the simplest case is considered to be constant, independent of space or concentration (Fickian diffusion; more general cases have also been considered, as we will note in appendix C.1). Thus diffusion is usually assumed to be a *smoothing* and homogenizing influence, that eliminates chemical gradients, and leads to uniform spatial distributions.

Turing and Diffusion-Driven Instability

This assumption was shattered in an unexpected and highly original paper, entitled 'The Chemical Basis of Morphogenesis' [354], published in 1952 by Alan Turing. He proceeded from the observation that interacting chemicals in a reacting system are everywhere coupled both kinetically and by diffusion, and noted that diffusion introduces a *spatial vector* into the system, in addition to the temporal vector embodied in the time evolution, which thus introduces the

potential for the formation of spatial as well as temporal asymmetries. Essentially, he hypothesized, and verified in his analysis, that chemical kinetics are possible for which diffusion could act as a *destabilizing* influence; that an initial homogeneous equilibrium, or uniform distribution of chemicals, which he termed **morphogens** (form-producers) in view of their suggested role in developmental pattern formation and morphogenesis (see chapter 4), could become unstable and develop stable nonuniform spatial patterns or structure, with the instability being triggered by small random spatially-dependent concentration perturbations present in any system at nonzero temperature. In terms of the general self-organization concepts examined above, such **reaction-diffusion systems** thus exhibit symmetry-breaking instabilities and the spontaneous generation of pattern, with the mechanism driving the instability being diffusion, which is counterintuitive in the light of its usual 'smoothing' property described above.

As we shall see, the generation of '*Turing structures*' out of a preexisting homogeneity has been amply confirmed, both by numerous analytical and numerical studies (see appendix A, and below) and by the recent experimental demonstration of steady-state spatially inhomogeneous structures maintained by diffusion (see appendix B.3.3). In the context of pattern formation and self-organization in developmental biology, Turing's seminal idea has proved to most fertile and productive, as the discussion of numerous models developed over the last twenty years, presented in chapter 4, will show.

Turing's Analysis To support his, at that time startling, claim of the destabilizing influence of diffusion, Turing [354] first discussed a simple numerical example, which demonstrated how concentration differences between two cells could be amplified by the flux of matter between them. He then examined mathematically with more rigour the breakdown of inhomogeneity through instability, by linearizing general reaction kinetics (that is, essentially assuming small concentration perturbations from a homogeneous equilibrium), and assuming only discrete diffusion between adjacent cells. He was able to obtain an exact solution to the resultant linear reaction-diffusion equations on a ring of N cells; he furthermore deduced mathematical relationships between the reaction rates (or combinations thereof) and the diffusion coefficients that were required for the different kinds of solutions to exist, and investigated the stability of these solutions.

Essentially, two major classes of solutions transpired to be possible, *stationary* and *oscillatory* solutions, each of which he classified further into special cases. The stationary solutions comprise stationary waves of the concentrations of the reacting chemicals around the ring, with a particular number of lobes or crests such that the wavelength divides into the circumference of the ring; the pattern for one 'morphogen' determines that of the other. In the oscillatory case, waves in the concentrations travel around the ring, so that there are two wave trains travelling in opposite directions; again the wavelengths are constrained to be submultiples of the ring circumference. In both the stationary and oscillatory cases, however, there is a '*chemical wavelength*' independent of the ring dimensions, and depending only on the kinetic and diffusion parameters, which is the limit to which the wavelengths tend as the ring is made larger. For further support for his thesis of the reasonableness of diffusion-driven instability, Turing finally presented a model chemical reaction system which displayed the averred behaviour. Comparing Turing's results with the insights of a general analysis into reaction-diffusion equations, as done below and in appendix A, we note that he was able to isolate and predict the main features of the solution behaviour of such systems.

Initial Responses Turing's work initially received little response and interest. One of the major objections was his use of linear rate equations, which were not chemically feasible — for example, they violated the obvious requirement of the non-negativity of solutions, a problem he 'solved' by imposing a cutoff at zero concentration. He was constrained to a linear analysis as such equations were the only ones he could solve analytically, the technique of high-speed computing not being available to him; although he did in fact present some numerical computations. In spite of this, Turing's actual equations did eventually receive some interest [19, 196, 197], as we shall see in the next chapter (section 4.3); but far more important was the fundamental *paradigm shift* he introduced through the realization of the symmetry-breaking properties of the appropriate interaction of kinetics with diffusion.

Thermodynamic Support: The Contribution of the Brussels School The concept of dissipative structure proposed by the Brussels school, as described at some length above (section 3.1.3), was first developed on purely thermodynamic grounds, independently of the above kinetic considerations. However, when these workers became aware of Turing's work, the set of reactions coupled with diffusion proposed by Turing was analysed thermodynamically by Prigogine and Nicolis [311], providing the link between the kinetic and thermodynamic approaches. They showed that near thermodynamic equilibrium, Turing's reaction scheme satisfied the requirements of linear nonequilibrium thermodynamics, and was thus thermodynamically feasible. Turing had taken all his reactions to be irreversible and thus infinitely far from equilibrium; they repeated his analysis more generally and confirmed the existence of an instability.

Most significantly, the existence of a well-defined symmetry-breaking critical affinity was demonstrated at large but finite distances from equilibrium, that is for a thermodynamically possible state; and that beyond the transition point, the homogeneous steady state was unstable, with the resultant formation of a dissipative structure in which the diffusion compensated the difference in reaction rates. In a later paper [310], the role of diffusion was clarified: although thermodynamically it increases the stability of the steady state, it also increases the manifold of perturbations compatible with the macroscopic equations of change; in the presence of diffusive spatial effects stability needs to be considered with respect to both inhomogeneous as well as homogeneous perturbations, leading to potential instability relative to nonuniform fluctuations. The analyses of Prigogine and coworkers thus gave a *theoretical underpinning* and support for the generation of spatial inhomogeneity in reaction-diffusion systems such as those first proposed by Turing, and hence validated the possibility of self-organization, the creation of structural and functional order, in open, dissipative chemical systems.

Almost simultaneous with this work, in the years around 1970, there was a heightened interest in periodic chemical reactions, whose existence was thus 'legitimized' by the above and analogous thermodynamic considerations (concerning both temporal and spatial symmetry-breaking). In particular, the observation of spatial and periodic order and structure in the Belousov-Zhabotinskii reaction, and the recognition of numerous biological periodicities (so-called 'biological clocks' and 'circadian rhythms') and biochemical oscillators, such as periodicities in the reactions of photosynthesis and glycolysis, led to intense renewed mathematical interest in the solution behaviour of nonlinear differential equations, and of the bifurcation or symmetry-breaking behaviour possible in their solutions [303] (a more detailed discussion of the Belousov-Zhabotinskii reaction, indicating its analysis by a mathematical, reaction-diffusion equation model, and the range of spatial and temporal symmetry-breaking, self-organizing so-

lutions latent in this system, is given in appendix B). By the early 1970s, the stage was set for an explosion of interest in reaction-diffusion equations, and the properties and applications of such systems to symmetry-breaking and self-organization.

Reaction-Diffusion Equations: Qualitative Behaviour

We have noted the potential self-organizing properties of the dynamical equations describing a system of chemicals which are both reacting and diffusing. The mathematical analysis of this class of equations has yielded a range of fascinating solution behaviours, an introduction to which is given in appendix A. We will not elaborate on the complexities inherent in the study of such systems, and refer both to the appendix, and to a range of useful references of varying degrees of mathematical sophistication, for example [5, 40, 87, 245, 251, 330]. In the following only a brief qualitative account of the main features of the solution behaviour will be given; we largely follow Peacocke [303] for this overview.

The kinetics and diffusion of a system of n interacting chemicals may generally be formulated mathematically by a system of equations of the form (see equation (A.1) and associated conditions)

$$\frac{\partial u_i}{\partial t} = f_i(u_1, \ldots, u_n) + D_i \nabla^2 u_i, \quad i = 1, \ldots, n; \tag{3.5}$$

where u_i is the concentration of the ith chemical species, and D_i is its diffusion coefficient. Here f_i represents the kinetic term, and includes the interactions between the chemical species that result in creation or destruction of the ith reactant. The second term in equations (3.5), which involves the space coordinates, in one, two or three space dimensions, includes spatial derivatives as it represents the time rate of change of u_i through (Fickian) diffusion (see section 4.2.1 for a discussion of diffusion). As the interactions f_i are in general nonlinear, the above equations (3.5) represent a system of *nonlinear parabolic partial differential equations*, which give rise to the mathematical complexities. Some aspects of the specific analytical properties of such equations are presented in appendix A.1, including some general conditions under which the existence and uniqueness of solutions to the equations (3.5) may be guaranteed. A specific feature of interest is the *stability* of solutions, as the long-term behaviour of the system can tend only to a stable solution in the presence of environmental perturbations.

The Basic Equilibrium Solution The fundamental solution that is sought in such a system is a homogeneous *steady state solution*, that is one in which all time variations $\partial u_i/\partial t = 0$, and there is no space dependence, so $\nabla u_i = 0$ (for $i = 1, \ldots, n$); for thermodynamically open systems, which permit the flux of matter and energy, such solutions are usually possible. The significant feature of reaction-diffusion and analogous systems, when the interactions are nonlinear, is that such a steady state solution, or solutions, may under appropriate circumstances become *unstable* at critical values of the controlling parameters present in the system (which may be the concentrations, assumed constant, of reagents in excess, the kinetic or diffusion coefficients, or parameters associated with the reaction domain size or geometry); a typical stability analysis, for a two-variable reaction-diffusion system, is given in appendix A.2.

Bifurcation to a Structured Solution Beyond such a critical value a new solution, or solutions, of the equations become possible, by the process of *bifurcation*, which refers to a

qualitative change in the topological structure of the solutions of a dynamical system, consequent on the shift in some critical parameter, λ. Some major features of bifurcations are discussed in appendix A.3; essentially, there is typically some critical value λ_c, such that for $\lambda < \lambda_c$ (or for some range in λ below the critical value), there is one asymptotically stable solution (such that all small perturbations from that solution decay back to it) — in terms of the analysis of the Brussels school, this constitutes the thermodynamic branch, the extension of the equilibrium solution — and for $\lambda > \lambda_c$, in some range above the critical parameter, two or more other solutions become possible and stable, instead of the one which characterizes the thermodynamic branch. The resultant possible solutions may exhibit organization in space and/or time, or can contain a multiplicity of stable solutions with accompanying hysteresis effects; we have the appearance of ordered 'dissipative structures'.

For reaction-diffusion equations, these structures may, as already indicated, embody a number of forms. We may firstly consider the equations in the absence of diffusion, as this corresponds to valid solution behaviour of reaction-diffusion equations with spatially independent concentrations. In this case, linear stability analysis as performed in the appendix indicates that the spatially homogeneous steady state may become unstable; this in itself does not indicate the presence of oscillations, but the application of certain theorems such as the Hopf Bifurcation Theorem quoted in the appendices (theorem A.10) for systems with only two intermediates may be used to determine whether or not stable *limit cycle* solutions, that is, isolated periodic orbits, will be obtained.

Sustained oscillations about steady states can occur only when the system is far from equilibrium, outside the 'linear' range, and are not possible at all for linear systems. A system in such an oscillatory regime moves around the limit cycle in a well-defined direction for a given set of parameters, so that a direction in time and a characteristic time scale, corresponding to the period of the oscillation, has been defined; that is, we have a *breaking of temporal symmetry*. This solution behaviour already corresponds to dissipative structure in the thermodynamic sense, here made explicit by the consideration of chemical kinetics which may give rise to such solutions (see for example the sustained oscillations observed in the Belousov-Zhabotinskii reaction described in appendix B, or in the model chemical oscillator, the 'Brusselator', whose behaviour is outlined below).

Spatial Structure But it is in the coupling of (oscillatory or non-oscillatory) kinetics to diffusion that the interesting and significant solutions of reaction-diffusion systems arise. In this case, spatial dependencies are introduced, and the possibility of space symmetry-breaking, and the formation of spatial structures, exists. For typical reaction-diffusion systems, such as those with no fluxes at the boundaries of the spatial domain, if there is only *one* concentration variable of interest, the first instability is determined by the chemical kinetics and no intrinsic spatial dependencies can be introduced, as is made more explicit in appendix A.1.4. But as soon as two or more chemicals are interacting, the spatial symmetry of the system may be broken: an intrinsic wavelength, determined only by the chemical kinetics and diffusion coefficients and not by the geometric constraints of the domain, is introduced. In this case, stationary and propagating waves in the concentrations of the reactants, including localized standing waves, and propagating solitary waves (in an infinite medium) are all possible; the temporal symmetry may also be broken through the introduction of limit cycles, and hysteresis effects due to the presence of multiple stable steady states may occur.

Extension to Higher Dimensions — Greater Complexity With three or more interacting state variables, even more possibilities arise. These include the potential for more than one region of instability in the parameter λ, and the interaction of different modes, with varying intrinsic wavelengths or frequencies, near critical values of λ, which may lead to 'cascading bifurcations', quasiperiodic or even chaotic behaviour. With the added possibility of varying more than one control parameter, diverse and complex forms such as rotating or spiral waves have been demonstrated to occur (all of these modes of behaviour have been observed in the Belousov-Zhabotinskii reaction, as indicated in appendix B). The potential for the irregular oscillations of chaotic behaviour, with characteristic scales varying from the microscopic to the macroscopic, is particularly interesting: it emphasizes again how the order of self-organizing systems is 'sandwiched' between the regime of thermal disorder, in which the presence of microscopic space and time scales leads to macroscopic homogeneity and equilibrium, and the opposite pole of nonequilibrium, turbulent chaos with a wider and larger characteristic range of scales [303].

Conditions for Pattern Formation in Reaction-Diffusion Systems

In the light of the above-mentioned range of possible solution behaviours for reaction-diffusion equations, it is well to consider the conditions under which the different forms of solution may occur. Such conditions have, for reasons mainly of analytic tractability, been derived only for the case of two state (concentration) variables, and follow from the *linear stability analysis* that yields conditions on the parameters for the homogeneous steady state to be stable to space-independent perturbations, but unstable to inhomogeneous fluctuations in the presence of diffusion. Such an analysis is demonstrated in appendix A.2; one of the earliest general analyses was performed by Segel and Jackson [319], and clear derivations emphasizing the main features may be found in the books by Murray [251] and especially by Edelstein-Keshet [87]. The most important concept to emerge from this analysis is that pattern formation is possible only under certain rather rigid conditions on the parameters of the system, or rather on dimensionless combinations thereof; the restrictiveness of these conditions for any given system subject to environmental perturbations may be a measure of its plausibility as a realistic pattern-forming mechanism [249].

In order to discuss the conditions for diffusion-driven instability, we briefly reproduce the relevant results derived in appendix A.2, using a slightly different notation: We consider a two-species reaction-diffusion system in one space dimension,

$$\frac{\partial u}{\partial t} = f(u,v) + D_u \frac{\partial^2 u}{\partial x^2}, \tag{3.6}$$

$$\frac{\partial v}{\partial t} = g(u,v) + D_v \frac{\partial^2 v}{\partial x^2}, \tag{3.7}$$

where D_u and D_v are the positive diffusivities of chemicals 1 and 2, with concentrations u and v respectively (which we have here not nondimensionalized), and the kinetic terms f and g are such that a positive homogeneous steady state (u_0, v_0) exists. For simplicity, we consider the equations on a finite domain, $[0,1]$ say after suitable scaling of the space variable, with zero flux boundary conditions. To demonstrate the presence of diffusion-driven instability, we linearize these equations about the homogeneous steady state (u_0, v_0), and derive the conditions on the parameters for the steady state to be stable to spatially homogeneous perturbations (that

is, effectively for $D_u = D_v = 0$), but unstable to some inhomogeneous perturbations. These conditions were shown to be (refer to inequalities (A.29), (A.30), (A.39) and (A.42))

$$\begin{aligned} a + d &< 0, && (3.8)\\ ad &> bc, && (3.9)\\ D_v a + D_u d &> 0, && (3.10)\\ (D_v a + D_u d)^2 &> 4 D_u D_v (ad - bc), && (3.11) \end{aligned}$$

where in the current notation, the Jacobian, or derivative, matrix has components

$$\begin{pmatrix} a & b \\ c & d \end{pmatrix} = \begin{pmatrix} f_u & f_v \\ g_u & g_v \end{pmatrix}_{(u_0, v_0)},$$

and all partial derivatives, for example $f_u \equiv \partial f / \partial u$, are evaluated at the homogeneous steady state (u_0, v_0).

The conditions (3.8)–(3.11) on the chemical kinetics may be explained in an elegantly simple way, as done in [319]. We note first of all that conditions (3.8), (3.9) and (3.10) together imply that

$$ad < 0 \quad \text{and} \quad bc < 0, \tag{3.12}$$

as the diffusion coefficients D_u and D_v are both positive. Hence one of the quantities a and d, but not both, is negative; similarly for b and c. We may relabel the species arbitrarily to choose $a = f_u > 0$, so that in consequence of the above sign requirements, the signs of the Jacobian matrix thus satisfy one of the following two forms [87]:

$$\text{Activator} - \text{Inhibitor} : \begin{pmatrix} + & - \\ + & - \end{pmatrix} \qquad \text{Positive} - \text{Feedback} : \begin{pmatrix} + & + \\ - & - \end{pmatrix} \tag{3.13}$$

Interpretation of the Instability Conditions We may now proceed with the interpretation of the conditions (3.8)–(3.11): The requirement, from (3.8), that one of a or d is negative — we have chosen this to be $d < 0$ — means that one of the chemicals, in this case chemical 2 with concentration v, inhibits its own formation; we call this substance an **inhibitor**. It follows from this condition and (3.10) that $a > 0$, as we have pointed out above; thus chemical 1 promotes or activates its own formation — that is, it exhibits autocatalysis — and is called an **activator** (Segel and Jackson's [319] initial terminology was 'stabilizer' and 'destabilizer' for activator and inhibitor; the current nomenclature was introduced *inter alia* by Gierer and Meinhardt [114]).

Furthermore, we note that one of b and c is negative, the other positive, giving two possibilities for the sign combinations as we noted above. The first case, with $b < 0$, $c > 0$, gives the first sign matrix above; here chemical 1 has a positive effect on its own synthesis and on that of chemical 2, whereas chemical 2 inhibits the formation of both substances, vindicating the labelling of this system as an 'activator-inhibitor' system. In the second case, labelled 'positive-feedback', the conditions $b > 0$, $c < 0$ indicate that each participant in the reaction system promotes an increase in chemical 1 and decrease in chemical 2. The term 'positive feedback system' in this case arises for historical reasons and should not be taken too literally [87], as both cases involve both positive and negative feedback loops; it is sometimes denoted instead as a 'cross activator-inhibitor model' [29], as opposed to the pure activator-inhibitor system described first.

The inequality (3.10) is equivalent to

$$\frac{D_v}{D_u}a + d > 0; \tag{3.14}$$

this condition combined with (3.8) and $a > 0$ implies that $D_v > D_u$, that is, the diffusion coefficients must be unequal, or more specifically, the inhibitor must diffuse through the system generally much more rapidly than the activator. This condition is crucial to the establishment of diffusion-driven Turing structures, and has proved to be the major hindrance in the experimental detection of such structures in chemical systems [308], as indicated in appendix B.3.3.

Furthermore, (3.8) is $a < -d = |d|$, so that if these parameters are taken as measures of the strength of activation and inhibition respectively, the inhibitory effect must be sufficiently strong relative to the autocatalysis to stabilize the homogeneous steady state. It is possible to define 'ranges' of activation and inhibition, as the mean distance between production and decay of molecules, which are derived based on the relative characteristic diffusion and kinetic time constants of these processes; in terms of these concepts, *the range of inhibition must be greater than the range of activation* for diffusion-driven instability to be possible.

Diffusion-Driven Instability: A Heuristic Explanation The above conditions have been obtained purely on the basis of mathematical considerations arising from the linear stability analysis, but they provide us with a general heuristic picture of how diffusion-driven instability is caused [114, 303]; we consider in particular the activator-inhibitor case: As a result of random concentration perturbations, at some location in the system a small local peak of activator concentration is produced. Due to autocatalysis, at that point the activator production accelerates and would cause a local autocatalytic explosion, were it not for the inhibitory effect which is sufficiently fast and strong to stabilize and reverse the process, as the activator growth also catalyzes an enhanced local production of the inhibitor. If we take diffusion into account, however, the inhibitor diffuses away from the local concentration maximum more rapidly than the activator, and so is unable to control fully the local activator production, which will increase until activator diffusion itself prevents localized confinement of the activator. In the meantime, the region surrounding the initial peak contains excess levels of inhibitor which prevent further peaks of activation, so that there is a minimum distance beyond which secondary peaks may be formed; this characteristic 'chemical wavelength' is defined by the interplay between the competing influences of activation and inhibition, and their respective diffusivities.

Local Activation and Long-Range Inhibition Thus the contrasting diffusion rates of activator and inhibitor result in a *local peak of activator* surrounded by an *inhibited region* within which further activation is impossible. We shall see that the qualitative behaviour exemplified here by chemical kinetics is far more widely applicable; such positive reinforcement on a local scale, together with a longer range of inhibition, is an underlying feature of a wide range of pattern-forming mechanisms associated with potential self-organizing behaviour, and is thus in general termed short-range (or local) activation and long-range inhibition, or more generally *lateral inhibition*; Oster [287] simply abbreviates the mechanism of local autocatalysis and lateral inhibition by LALI, a convention we shall also employ. We will encounter a range of such lateral inhibition models in the course of our study of self-organization in developmental biology, and discover that in spite of differences in the underlying physical mechanisms, essentially isomorphic

patterns are predicted, due to the fundamental similarities in the mode of operation of these mechanisms, as evidenced by the analogous mathematical formalism. For introductory overviews of lateral inhibition models, see for example [66, 89, 287].

Stationary Patterns in Reaction-Diffusion Mechanisms

The diversity of possible solution behaviours latent in systems of reaction-diffusion equations, ranging from temporal oscillations to stationary and travelling waves, and more complicated behaviour, has already been referred to; furthermore, we have considered above some of the conditions on the reaction kinetics for such patterning behaviour to be possible for two-variable systems. Here we will just point out briefly some aspects of stationary spatial patterns arising in two coupled reaction-diffusion equations, and refer to appendix A.4 for more details.

The Interplay between Kinetic and Geometric Scales As already emphasized, the interaction between kinetics and diffusion gives rise to a characteristic spatial scale, the 'chemical wavelength', embodying the macroscopic correlations in the self-organized system. For interactions occurring in a finite domain, the boundary conditions imposed by the geometry and scale of the system introduce further constraints on the scales of the patterns, and the final scaling is the consequence of a balance between these two, not necessarily compatible, requirements.

In brief, the geometric constraints result from the need for a fixed, integral number of spatial wavelengths to fit into the domain, subject to the boundary conditions, which are most frequently chosen to be 'zero flux' conditions, corresponding to a closed system with no inflow or outflow of reactants. The chemical wavelength, on the other hand, arises mathematically from the linear stability analysis (chemically, it corresponds to the scale of the inhibited region — see above), and indicates the mode of the spatial perturbations which will be amplified most rapidly in the presence of diffusion. The wavelength of the final standing waves (in a one-dimensional system), at least some distance after instability, then corresponds to a compromise, acceding as far as possible to the chemical wavelength, but usually slightly modified so as to fit a whole number of peaks and troughs into the domain.

We have so far mentioned simply 'standing waves'; this is justified as, in fact, the pattern that is formed after the bifurcation point is, to first order, characteristic purely of the domain (more technically, bifurcation theory indicates that the bifurcating solution is approximated by the eigenfunctions of the Laplacian operator on the domain geometry and with the appropriate boundary conditions — see appendix A.3), and on a one-dimensional line of reaction, the pattern is essentially cosinusoidal, thus indeed yielding the familiar standing wave pattern with peaks or troughs at the ends. As is discussed in more depth in the appendices, this result holds best near the point of instability and where a small number of waves fit into the domain (that is for small wave numbers); beyond this range, nonlinear effects and superposition of modes are liable to dominate.

Size Dependence of Patterns A consequence of the interplay between domain geometry and chemical wavelength is a significant *size dependence*. If the domain is too small, of length less than half the chemical wavelength, no pattern at all may be formed, in spite of the satisfaction of all kinetic requirements such as (3.8)–(3.11); there is a *critical domain size* for the establishment

of dissipative structure. For slightly larger domains, half a cosine wave will fit into the region, leading essentially to a **gradient** of the reacting chemical species, and an intrinsic polarity; the potential biological significance of this will become apparent in section 4.2. Even larger domain sizes will then permit one or more complete cosine waves at or near the chemical wavelength to satisfy the boundary conditions, so that multiple peaks and troughs correspond to periodic chemical patterns; for applications to development see section 4.3.

It may in fact occur that there are domain sizes beyond the minimum size for the establishment of a gradient, for which no pattern is formed due to a failure to match the chemical wavelength and boundary conditions; this situation is demonstrated well numerically by Eilbeck [93]; see also [44, 199]. It is furthermore apparent that in a growing domain, one is liable to observe a succession of patterns, with increasingly more crests and troughs, as more and more multiples of the basic chemical wavelength can fit into the region; this situation was studied, together with an investigation of the modes that dominate, by Arcuri and Murray [3] (see also [180]).

Two-Dimensional Patterns In one space dimension the possible patterns are clearly rather limited, to gradients and periodic patterns. The progression to two dimensions immediately introduces extra patterning potential and associated complications. For a rectangular domain, the solutions expected by linear theory are independent cosinusoidal waves in the two directions, but nonlinear interactions between these waves tend to distort the pattern. Nevertheless, numerical investigations tend to reflect spotted patterns or stripes; the choice between these depends strongly on the shape of the domain, with stripes frequently occurring when the domain geometry is narrow, and quasi-one-dimensional patterns are formed, as is established in appendix A.4. Note however that there appear to be classes of reaction-diffusion systems with a preference for stripe formation (see [211, 263]). Other domain shapes, such as circular or elliptical geometries, and the extension to three dimensions in particular, give rise to a wider variety of possible patterns; these are however always to some extent characteristic of the domain and boundary conditions (being based on eigenfunctions of the Laplacian operator); and display analogous interactions between the chemical wavelength and the geometry, and similar dependence on domain size.

Nonlinear Effects Further numerical investigations of the behaviour of specific reaction-diffusion models have revealed that they fall into two general 'families', with respect to their *nonlinear* responses to changes in system size [150], providing effects that could not have been deduced from linear theory alone. The detailed models which exemplify the different behaviours will be introduced later in this thesis, and an outline of the differences is given in appendix A.4.3. We thus here only state briefly that the 'Brusselator' model [310] (see section 3.2.3) adapts readily to changes in the domain size, showing a preference for maintaining the intrinsic chemical wavelength derived from linear theory. The 'Gierer-Meinhardt' model [114] (see section 4.2.2), on the other hand, produces robust patterns that tend to be maintained on alterations in system size or parameters. Other reaction-diffusion systems may broadly be classified into one of the above two classes of behaviour, thus providing a valuable means for discriminating between mechanisms applied in developmental situations, to expedite the comparison with experiment [150].

Methods of Investigation The means of investigation of such systems have included *analytical methods* such as linear stability analysis, bifurcation theory and asymptotic techniques, some of which are demonstrated in the appendices, and the results of which are summarized above. However, the intrinsic nonlinearities in the reaction-diffusion equations of interest impose fundamental limitations on the accuracy and predictive power of these analytic methods, especially for large domains or those with complicated shapes (particularly in more than one dimension), and for situations some distance away from the symmetry-breaking instability. For such cases, and in general to corroborate what clearly are just analytical approximations, it is desirable to turn to *numerical techniques*, and some of those which have been applied to the study of reaction-diffusion equations are described briefly in appendix A.5. These investigations have yielded a range of patterns, which are in many ways reminiscent of situations in chemistry, biology or ecology, and which have thus been proposed as plausible bases for self-organization in 'real' systems; we will consider and evaluate a range of such 'explanations' in developmental biology in the next chapter.

In spite of considerable mathematical activity (see for example [5, 40, 101, 330]) and numerous numerical simulations, the investigations of the pattern-forming potential of reaction-diffusion systems have been somewhat limited. For example, only the situation of strict self-organization from an initially homogeneous background and steady state has been emphasized; indeed, as we have seen, the potential of systems of interacting and diffusing chemicals for such self-organization underlies their importance in the theoretical study of the generation of dissipative structure, and their status as a paradigm for self-organization.

We shall however have cause to cast doubt on the assumption of strict homogeneity in any biological system; and even if this were ever valid, reaction and diffusion can clearly also occur in a system with preexisting environmental inhomogeneities. Thus it is somewhat surprising that the first analysis of a simple space dependence of one of the diffusivities has only been published rather recently [29], and that the number of other studies including space dependence of reaction terms and other parameters seems to be rather limited (see for example [6]). The self-organizing, symmetry-breaking potential of reaction-diffusion systems has been amply confirmed and verified since Turing's pioneering investigations [354], but the study of the solutions to such equations under more general conditions on the parameters is, in a sense, only beginning.

3.2.3 Chemical and Biochemical Examples of Reaction-Diffusion Systems

In the above analysis, the range and diversity of solutions possible in even a two-variable reaction-diffusion system was indicated, and some features of the possible patterns and the parameter ranges within which they could occur, were pointed out. From the aforegoing study, it is however not yet clear whether *realistic* kinetic schemes which exhibit the desired temporal and spatial pattern-forming behaviour exist, or whether our consideration only of two-variable systems, while analytically tractable, is not an unrealistic simplification. To clarify this issue, we consider some plausible chemical and biochemical schemes which may be modelled by a two-variable reaction-diffusion system.

Enzymatic Self-Organization

A wide variety of biochemical and biological oscillators is now known, for example in glycolysis, and so it was necessary and inevitable that models were proposed for the understanding of such oscillations, which constitute temporal self-organization. A multitude of such models is now available, many of which involve enzymatic reactions. The full kinetic schemes for such reactions are generally fairly intricate, but may often be simplified considerably if, for example, the rates of decomposition of most intermediate complexes are fairly large, or the enzymes are present in very small amounts. In such cases the kinetic behaviour may be reduced to a small number of critical intermediates and rate-limiting steps, and may then be formulated in terms of reaction equations, possibly including diffusion, in a few variables. An introductory discussion, with numerous examples, of the different types of enzymatic reaction schemes that may give rise to two-variable kinetics displaying temporal oscillations is given by Peacocke [303, pp.161–171].

Possible mechanisms for such schemes include *end-product inhibition*, where an early stage of an enzymatically catalyzed reaction sequence is inhibited by the end-products of the sequence; *substrate inhibition*; *activation by product*, where one of the reaction products of an enzyme sequence activates an enzyme earlier in the chain and hence stimulates its own synthesis and that of its precursors; *cooperativity* in allosteric enzyme action; and the *reversible covalent modification* of an enzyme. All these mechanisms involve feedback loops ultimately triggering autocatalysis or self-inhibition, and thus giving rise to nonlinear terms in the reaction kinetics; and, as has been shown by numerous authors, and summarized in [303], all show the potential for temporal self-organization.

In the presence of diffusion, models for allosteric enzymes, with applications to the phosphofructokinase reaction in the glycolytic cycle [121], and substrate inhibition involving negative feedback control [238], have been demonstrated to have the potential not only for undamped oscillations, but also for the formation of finite amplitude spatial structures; this is demonstrated through their formulation in terms of two-variable reaction-diffusion systems. A further interesting phenomenon which has been demonstrated in substrate inhibition oscillators, is the possibility of stable solitary travelling waves in the concentration of one of the reagents, at a wave speed orders of magnitude faster than typical transport times obtained through a pure diffusion process. If cell walls are permeable to one of the substrates, this wave has been shown to have the property of passing through cell walls, thus possibly triggering an avalanche effect due to the threshold nature of the kinetics; such a wave arising through a reaction-diffusion mechanism provides a potential means for the transmission of biochemical signals between cells, hence constituting a basis for rapid information flow between different parts of an organism [42].

A Model Immobilized Enzyme System An enzymatic system which has been studied in some detail, both experimentally and theoretically, is one in which the enzyme is immobilized in an artificial membrane structure [188]. Such physical confinement of enzymes, while permitting the substrates to diffuse through the membrane, allows the controlled study of the enzymatic reactions and diffusion. The reactions considered consume two substrates, S and A, and are inhibited by excess S and activated by A; they thus also fall into the class of substrate inhibition. One application is in the phosphofructokinase reaction, but the simplest application is one in which the (immobilized) enzyme uricase is a catalyst for the reaction

uric acid + oxygen → allantoïn + other products.

The dimensionless form of the empirical rate equations, including diffusion, for the substrate uric acid (s) and cosubstrate oxygen (a) can be written as

$$\frac{\partial s}{\partial t} = s_0 - s - \rho F(s,a) + \nabla^2 s, \tag{3.15}$$

$$\frac{\partial a}{\partial t} = \alpha(a_0 - a) - \rho F(s,a) + d\nabla^2 a, \tag{3.16}$$

$$\text{where} \quad F(s,a) = \frac{sa}{1 + s + Ks^2}, \tag{3.17}$$

and ρ, α, s_0, a_0 and K are positive constants.

The interpretation of these equations is basically that s and a are supplied at constant rates s_0 and αa_0, degrade linearly proportional to their concentrations, are used up in the reaction at a rate $\rho F(s,a)$, and diffuse according to Fick's law. The form of $F(s,a)$ exhibits substrate inhibition, in that for a given a, $F(s,a)$ has order $O(sa)$ for small s and thus increases linearly with s (in fact, for low s the uptake F has a Michaelis-Menten form), while for s large it is $O(a/Ks)$, and decreases with s; that is, excess substrate inhibits the rate of the reaction, and the parameter K is a measure of the severity of the inhibition.

This substrate inhibition scheme has been investigated in detail, as already pointed out, using a wide range of analytical and numerical techniques; and the interaction of enzymatic kinetics and diffusion has been demonstrated to produce macroscopic spatial, temporal and functional structures in a self-organized way. Types of solutions that have been shown to occur include multiple steady states and hysteresis, oscillations, stationary spatial pattern formation and wave front propagation [189, 190]. A detailed analysis of this system and techniques used in its study as an example of self-organization phenomena is given in the book by Kernevez [188]; we shall return to this system with an application to animal coat patterning [248] (see section 4.3.2).

A Model Chemical Reaction System — the Brusselator

A heuristic explanation of the various terms in a two-variable reaction-diffusion equation system may usually be given, in terms of mutual activating and inhibiting effects of the reactants; as was done for example for the above substrate-inhibition system. It is another matter entirely to exhibit a complete and realistic set of elementary chemical reactions whose kinetics reduces to such a simple two-variable system.

In order to obtain a reaction sequence whose kinetic equations display the desired properties, including oscillations, spatial structure in the presence of diffusion, and the analytic tractability of being reducible to a two-equation system, we consider a chemical system involving two variable intermediates, together with a number of initial and final products whose concentrations are assumed to be controlled throughout the process. For such systems, it has been shown (see for example [357]) that limit cycle solutions are impossible in a reaction sequence involving only uni- and bimolecular elementary reaction steps. Hence we are constrained to include at least one *trimolecular* step in the reactions to obtain self-organizing solutions.

The Brusselator: Basic System Formulation A model chemical reaction system which displays chemical instability and the associated generation of spatial and temporal dissipative structure is the much-studied 'Brusselator', named in view of its development by the Brussels group, and introduced by Prigogine and Lefever [310]. The basic reaction scheme is given by

$$\mathrm{A} \xrightarrow{k_1} \mathrm{X}, \tag{3.18}$$

$$\mathrm{B} + \mathrm{X} \xrightarrow{k_2} \mathrm{Y} + \mathrm{D}, \tag{3.19}$$

$$2\mathrm{X} + \mathrm{Y} \xrightarrow{k_3} 3\mathrm{X}, \tag{3.20}$$

$$\mathrm{X} \xrightarrow{k_4} \mathrm{E}. \tag{3.21}$$

The overall reaction is

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{D} + \mathrm{E}, \tag{3.22}$$

and all the equations are taken to be irreversible for simplicity (that is, the system is infinitely far from thermodynamic equilibrium; this may be achieved by the removal of the reaction products D and E as soon as they are created).

Here the elementary reaction (3.20) is the obligatory autocatalytic, trimolecular step. Such trimolecular reactions are extremely rare in chemistry, if indeed they occur at all, so that such a model might appear unrealistic at first sight; but as we have seen above, more complicated higher order systems involving, for example, enzymes may reduce to a two-variable system with a nonlinearity similar to the one introduced by the trimolecular step. Thus the analysis of the Brusselator is justified as a convenient and simple, if in itself implausible, model for a range of realistic mechanisms with an analogous mathematical formulation. In the light of the requirement for trimolecularity for the existence of limit cycle solutions, it was for a number of years indeed thought that the Brusselator was the simplest mechanism displaying oscillatory properties [357], but a class of simpler trimolecular two-species models has been discovered by Schnakenberg, requiring only three elementary reactions [317].

To formulate the kinetic equations we consider the concentrations A, B, D and E to be effectively constant, so we deal effectively only with the variable intermediates X and Y, and take diffusion into account. This leads to the two-variable system of reaction-diffusion equations

$$\frac{\partial X}{\partial t} = k_1 A - k_2 B X + k_3 X^2 Y - k_4 X + D_X \nabla^2 X, \tag{3.23}$$

$$\frac{\partial Y}{\partial t} = k_2 B X - k_3 X^2 Y + D_Y \nabla^2 Y. \tag{3.24}$$

The formulation of the differential equation system is completed with the specification of boundary conditions, for example zero fluxes at the boundaries of the domain, and initial conditions, which may be random perturbations from the homogeneous steady state we derive below. According to our previous studies of reaction-diffusion equations, we expect this system to exhibit various kinds of cooperative, symmetry-breaking behaviour. This possibility has been demonstrated in general in appendix A; we here outline the analysis for this particular case, as an example of the basic techniques used for the analysis of such a reaction-diffusion system (see [87, 251]).

Nondimensionalization The reaction-diffusion system (3.23)–(3.24) depends on four kinetic parameters, two constant concentrations, two diffusivities and the dimensions of the system, in a way that, as we shall see, involves combinations of these parameters. Better insight into the qualitative behaviour may be obtained if we *nondimensionalize* by introducing scaled variables; in the process we also reduce the number of free parameters. We introduce a characteristic length scale L, which may be related to the dimensions of the system, and the variables

$$t^* = k_4 t, \quad \mathbf{x}^* = \mathbf{x}/L, \quad D_u = D_X/k_4 L^2, \quad D_v = D_Y/k_4 L^2,$$

$$U = \left(\frac{k_3}{k_4}\right)^{1/2} X, \quad V = \left(\frac{k_3}{k_4}\right)^{1/2} Y, \quad a = \left(\frac{k_1^2 k_3}{k_4^3}\right)^{1/2} A, \quad b = \frac{k_2}{k_4} B.$$

In these variables, and dropping the superscripted stars, one finds that the reduced variables U and V satisfy the system

$$\frac{\partial U}{\partial t} = a - (b+1)U + U^2 V + D_u \nabla^2 U, \tag{3.25}$$

$$\frac{\partial V}{\partial t} = bU - U^2 V + D_v \nabla^2 V. \tag{3.26}$$

(Note that these nondimensionalized equations are chosen to correspond to the published work on the Brusselator, and are slightly different from the general form in appendix A.2; the equations (3.25)–(3.26) may be put in the general form (A.19)–(A.20) by the definition of the nondimensional parameter $\gamma = L^2 k_4/D_X$, and the slight change in the definitions of the variables $t^* = D_X t/L^2 = k_4 t/\gamma$, $d = D_Y/D_X$; D_u and D_v are then no longer required.)

In thermodynamic equilibrium (that is, for fully reversible reactions) this system admits a single, spatially homogeneous, stable steady state at $U = a$, $V = b/a$, at which point all space and time variations vanish. As we gradually increase the overall affinity of the reaction and continuously drive the system away from equilibrium, this uniform solution remains, and corresponds to the 'thermodynamic branch'; but far from equilibrium, however, and especially in the irreversible limit, such a solution is no longer necessarily stable, depending on the nature of the perturbations it is subjected to, and the values of the parameters and diffusivities in the equations.

Linear Stability Analysis In order to assess the equilibrium of the steady state, and investigate the possibility of cooperative behaviour, we perform a *linear stability analysis*, as discussed more fully in appendix A.2; the motivation for such a study, as we have seen, is that a solution is (asymptotically) stable if sufficiently small perturbations decay, and unstable if an arbitrarily small deviation from the solution grows with time and changes the qualitative behaviour completely. Essentially, stable solutions are robust, whereas unstable solutions will never be observed in the presence of inevitable concentration fluctuations. The analysis is simplified and made more transparent if we restrict ourselves to one space dimension, with coordinate x.

Thus we assume small perturbations u and v from the (normalized) steady state concentrations, that is

$$U = a + u,$$
$$V = b/a + v,$$

where $|u| \ll 1$, $|v| \ll 1$. Retaining only linear terms, we obtain from (3.25)–(3.26):

$$\frac{\partial u}{\partial t} = (b-1)u + a^2 v + D_u \frac{\partial^2 u}{\partial x^2}, \tag{3.27}$$

$$\frac{\partial v}{\partial t} = -bu - a^2 v + D_v \frac{\partial^2 v}{\partial x^2}. \tag{3.28}$$

Now it may be shown by direct substitution that solutions having the form $u = u_0 e^{\sigma t + ikx}$, $v = v_0 e^{\sigma t + ikx}$ satisfy this linear system provided the temporal eigenvalue σ and the spatial 'wave number' k are chosen appropriately. Thus if we assume perturbations of that form, and substitute into equations (3.27)–(3.28), we obtain the condition for nontrivial solutions for u_0 and v_0 to be

$$\sigma^2 + \sigma(a^2 - b + 1 + (D_u + D_v)k^2) + (D_u D_v k^4 + (a^2 D_u + (1-b)D_v)k^2 + a^2) = 0. \tag{3.29}$$

For linear stability, we require Re $\sigma_{1,2} < 0$ for the two roots of this quadratic equation, so that conditions for *stability* are

$$(D_u + D_v)k^2 + a^2 - b + 1 > 0 \tag{3.30}$$

and

$$D_u D_v k^4 + (a^2 D_u + (1-b)D_v)k^2 + a^2 > 0. \tag{3.31}$$

Temporal Symmetry-Breaking and Oscillations From this analysis, some of the possible solution behaviour of the Brusselator may immediately become apparent. We may firstly consider the spatially homogeneous situation: If the diffusion terms are suppressed, that is $D_u = D_v = 0$ — or alternatively, if we consider space-independent perturbations, corresponding to $k = 0$ or infinite wavelength fluctuations — then the inequality (3.31) is automatically satisfied, but from (3.30) we obtain the result that the steady state becomes unstable (to spatially homogeneous perturbations) if

$$b > a^2 + 1. \tag{3.32}$$

Under these conditions the time-independent equilibrium will no longer be observed. Further investigation, using bifurcation techniques, indicates that at this critical value of b, a Hopf bifurcation occurs (see appendix A.3), and the system undergoes undamped periodic oscillations, with a well-defined period independent of the initial conditions, which may be approximated using asymptotic methods. The resultant solution behaviour is a limit cycle, which heralds the breaking of temporal symmetry, the introduction of a characteristic time scale and the creation of temporal structure — that is, temporal self-organization. The system behaves as a chemical clock in a markedly coherent manner, and this simple model, the Brusselator, may thus be used as a paradigm for the entire range of chemical oscillations, including the Belousov-Zhabotinskii reaction (see appendix B).

Spatial Dissipative Structure While temporal oscillations are interesting, we seek especially the possibility of the generation of spatial dissipative structure. To investigate potential spatial inhomogeneities, we now no longer neglect diffusion in our stability analysis; but consider still the concentrations A and B and the parameters as space-independent. Now, in addition to the

above-mentioned possibility of temporal symmetry-breaking at $b = 1 + a^2$, the inequality (3.31) provides a condition for instability with respect to spatial perturbations, for a given k:

$$b > \frac{D_u D_v k^4 + (a^2 D_u + D_v)k^2 + a^2}{D_v k^2}. \tag{3.33}$$

By minimizing the right-hand-side of (3.33), we may obtain the critical wave number at which instability first occurs (for the smallest b; we have followed the tradition of analysis of the Brusselator and chosen b as the bifurcation parameter [310, 276]); in terms of the (reduced) wavelength $\lambda = 1/k$, this gives a critical wavelength for the onset of instability, λ_c. The physical meaning of this wavelength is best appreciated in the original dimensional variables; it is given by

$$(\lambda_c)^2 = \left(\frac{k_4}{k_1^2 k_3}\right)^{1/2} \frac{(D_u D_v)^{1/2}}{A}, \tag{3.34}$$

and corresponds to a critical concentration of the reacting species B at which the spatial symmetry-breaking bifurcation occurs:

$$B_c = \left[\frac{k_1}{k_4}\left(\frac{k_3}{k_2}\frac{D_X}{D_Y}\right)^2 A + \left(\frac{k_4}{k_2}\right)^{1/2}\right]^2. \tag{3.35}$$

The significance of the above results is immediately apparent. There is a 'chemical wavelength', which depends intrinsically on a combination of the kinetic parameters, diffusivities and precursor concentrations, which establishes a characteristic spatial scale in the system. The previous spatial homogeneity of the system is broken, and spatial structure may arise spontaneously, under conditions which are far from equilibrium: we have confirmed the *potential for self-organization*, for the *creation of dissipative structure*, in this ostensibly simple chemical system; and hence opened the way for the search for similar ordered structures in the more complicated systems common in chemistry and biology.

Analysis beyond the Bifurcation Point The analysis of the Brusselator model beyond the bifurcation point is considerably more complicated, as any analyses must take into account the *intrinsically nonlinear* nature of the model equations; for an outline of some of the analytical methods that may be utilized to proceed, see appendix A.3. We shall here not attempt to present any of these methods or the results that have obtained, except to indicate very briefly some of the studies that have been performed.

In the neighbourhood of the primary bifurcation point, perturbation techniques have been employed to construct first-order approximations to the spatially heterogeneous solutions. Auchmuty and Nicolis outlined their results in [275], and presented a fuller description in [6]: By the use of bifurcation analyses, they constructed the bifurcation diagram and the new steady state solutions arising after bifurcation (for both fixed Dirichlet and zero flux Neumann boundary conditions), and confirmed the stability of these solutions. The studies were extended to the situation of an inhomogeneous medium, where the precursor concentration A was nonuniform; in this case, the presence of *spatially localized* dissipative structures, and the possibility of standing and propagating concentration wave solutions, was demonstrated. The results obtained analytically were confirmed through numerical simulation by Herschkowitz-Kaufman [160]. The bifurcation analyses were also extended to oscillatory solutions in [7]. An account of the techniques

used and results obtained is presented by Nicolis and Prigogine [276], with a more descriptive introduction being presented by Peacocke [303].

Of the other studies that are available, we may mention the work of Keener [181], who applied the method of two-timing to study *secondary* bifurcations for a general system of two coupled reaction-diffusion equations, with specific application to the Brusselator; the types of secondary bifurcations in this model were classified. As an example of the numerical analyses that have been performed, we point out that Kubíček *et al.* [193] studied the continuous dependence of spatially nonuniform concentration profiles on the characteristic length of the system. The work mentioned does not nearly cover the range of studies that exists for the Brusselator, but serves only to highlight that even for such a seemingly simple and idealized chemical reaction system, extensive and intricate analyses, requiring the full power of the mathematical machinery of bifurcation and perturbation techniques, are needed to elucidate the diversity of solution features inherent in reaction-diffusion systems.

Thermodynamic Analysis We note in concluding our brief discussion of the Brusselator as a paradigm for chemical self-organization and the reaction-diffusion kinetic formalism, that the original introduction of the Brusselator scheme to the literature [310] was accompanied by a stability analysis based on the results of nonlinear far-from-equilibrium thermodynamics, to show that the instability was not merely a construct of the kinetic equations. In brief, the excess entropy production $\delta_X P$ referred to above, in section 3.1.3, in this case becomes

$$\delta_X P = \frac{1-b}{a}u^2 + \frac{a^3}{b}y^2 + \frac{D_u}{\lambda^2 a}u^2 + \frac{D_v a}{\lambda^2 b}v^2, \tag{3.36}$$

where again u and v are small perturbations, with reduced wavelength λ, from the homogeneous steady state $U = a$, $V = b/a$. For instability, the evolution criterion requires $\delta_X P < 0$. We note that this criterion must be satisfied through the only negative contribution to the excess entropy production in (3.36), the term $-(b/a)u^2$, due to the autocatalysis of reactant X. In terms of our previous considerations, this is as we would expect, as the 'dangerous' contributions to chemical reaction mechanisms, giving rise to instability, must arise from autocatalytic or cross-catalytic effects.

We note here that the diffusive contribution to the excess entropy production is given by the last two terms, and thus is positive; thus, as we would expect, in thermodynamic terms diffusion acts to stabilize the steady state by smoothing out perturbations. On the other hand, by introducing a spatial vector into the system, diffusion increases the manifold of perturbations compatible with the macroscopic equations of change which are capable of destabilizing the system, by permitting also inhomogeneous perturbations, and thus ultimately allows the formation of the spatial symmetry-breaking instability, leading to inhomogeneities.

Analyses such as the above confirm that the kinetic theory of bifurcations based on the solution of dynamical equations such as reaction-diffusion equations, and the results of the thermodynamic analyses, agree fully on the origin and nature of symmetry-breaking instabilities, leading to the self-organization of dissipative structures [310]. The preceding discussions should however have made it clear that the thermodynamic approach can only indicate the *potential* for instability, and give general, phenomenological indications of its nature and location. The dynamical, kinetic analyses, on the other hand, are far more powerful, delineating the parameter domains in which different types of solution behaviour are possible, and, when combined

with bifurcation and asymptotic techniques and with numerical analyses, also giving a good description of the characteristics, such as polarities, symmetries and patterns, of the dissipative structures beyond equilibrium. Thus it will henceforth be solely to dynamical analyses that we will turn when studying self-organization and pattern-forming behaviour in dissipative systems in developmental biology.

3.3 A Model Biological System — The Slime Mould

The preceding sections have laid much emphasis on self-organization *per se*, concerning both the physical concept, and its mathematical study within the framework of dynamical systems. Our major goal is to apply such concepts to detailed models of aspects of pattern formation and morphogenesis in multicellular eucaryotes. As is clear however from our brief excursion (chapter 2) to glimpse at some of the pertinent biological background, the developmental processes are highly intricate, with a diversity of genes involved in the regional specification mechanisms, and numerous molecular and cellular species participating in a complicated fashion in the patterning of tissues and organs. Before we proceed to the higher organisms, therefore, it is again profitable to outline a comparatively 'simple' model system, for which a relatively complete dynamical understanding is being approached, in lieu of obtaining a detailed understanding of multicellular organisms, which is far from feasible at present. The hope is as usual that the processes recognized in this model system may form the mechanistic building blocks from which more complex structures are constructed.

We have already briefly encountered the cellular slime mould, which displays the rudiments of morphogenetic behaviour, and forms a biological paradigmatic case of self-organization, fulfilling the same role as the Belousov-Zhabotinskii reaction does for chemistry (see appendix B). As we might expect, models of slime mould behaviour are even more complex and intractable than those for the BZ reaction. Most studied and best understood is *Dictyostelium discoideum*, whose life cycle we shall briefly sketch below; but it should be noted that other species of slime mould (which fall into two phyla, the cellular slime moulds, Acrasiomycota, and the plasmodial slime moulds, Myxomycota) have also been modelled and show slightly different behaviour — another much-studied case is *Polysphondylium pallidum* (see [66] and references therein). Elementary descriptions of the slime mould life cycle and modelling thereof are found in numerous works, for example [10, 251, 277]. The book by Bonner [35] gives a comprehensive summary of the early work, in particular of the mechanisms of migration.

Outline of Life History

The cellular slime moulds pose a semantic difficulty about what it means to be an 'individual organism'. In its native, feeding stage, the population consists of hundreds or thousands of unicellular amoeboid cells, each moving independently on, usually, a forest floor, engulfing bacteria by phagocytosis, and reproducing sexually or by asexual division. The population density is essentially constant, such that the individual cells form a globally uniform system. When nutrient is depleted, however, a starvation phase ensues, in which a coordinated sequence of events leads to the formation of a multicellular fruiting body with two types of differentiated cells.

The complex sequence of events triggered by a scarcity of food appears to begin, after a few

hours, with a few 'pacemaker' cells emitting periodic signals of cyclic adenine monophosphate (cAMP) into the medium. Such pulses of cAMP are propagated every few minutes, are relayed by the receiving cells, and stimulate the aggregation of cells towards the foci of aggregation, the pacemaker cells. The amoebae are attracted by the cAMP, and move *chemotactically* (in response to the chemical stimulus) towards the signalling centres; the motion shows signs of self-organization, being highly coordinated spatially and temporally, and circular and spiral wave patterns of aggregating motion are observed. As cells congregate, contacts form, and eventually up to a hundred thousand amoebae may coalesce into a shapeless multicellular mass around a given pacemaker cell.

The aggregate next undergoes a series of contortions and shape changes; a conical mass rises from the cell bulk, continues to elongate and eventually topples over like a slug. This sluglike collection of cells may undergo a crawling motion towards a more favourable environment, while simultaneously periodic wavelike contractions occur in the slug body. Already at this stage, a process of differentiation has begun; the cells in the anterior (front) portion are biochemically somewhat different from those in the rear, with anterior cells eventually becoming stalk and the posterior, spore cells, while the ratio of the two cell types is constant and self-regulating in the face of surgical manipulation. Finally the slug stops moving, and a sequence of shapes is created, culminating in the formation of a slender stalk, surmounted by a spore-filled capsule, the fruiting body. The final multicellular organism is thus strictly differentiated into spore and stalk cells — although they were derived from the same amoebae, they are different chemically, physically and functionally. In order to provide a rigid structural basis, the stalk cells harden and die, while the spores are protected against the harsh conditions. When environmental conditions become more favourable, the fruiting body splits open and releases the spores, which are dispersed by air currents and, if landing in suitable surroundings, begin feeding to complete the cycle and propagate the species.

In this repertoire of events, we have thus witnessed a rudimentary developmental process, involving *pattern formation* (for instance in the distribution of aggregating cells, and the commitment to pre-spore and pre-stalk cells in a fixed ratio), *differentiation* into different cell types, and *morphogenesis*, the birth of form such as of the fruiting body atop the stalk. The developmental system is readily studied, being easily accessible and malleable, and has consequently long been a favourite experimental organism, so that many of the biochemical and morphological changes are known in detail. Nevertheless, in spite of the comparative simplicity relative to other species of developmental interest such as nematodes, insects or vertebrates, the chemical and genetic underpinnings of *D. discoideum* self-organization and development are highly complex, with at least fifty genes being directly responsible for (and perhaps another hundred peripherally involved in) the successful operation of the aggregation phase alone [303].

A Brief Overview of Models of Slime Mould Development

A range of interesting theoretical issues arises in the dynamical study of slime mould development; some of these problems are [87]:

- How is the aggregation initiated and coordinated in its coherent spatiotemporal patterns?
- What mechanisms underlie slug locomotion?

- How is prespore-prestalk commitment determined, and the ratio between the differentiated cell types controlled?
- What forces regulate the morphogenetic processes that form a variety of shapes, including the final *sporangiophore* (spore-bearing structure)?

In the quest for at least a partial understanding of the developmental dynamics involved, detailed modelling has been attempted, with considerable qualitative and quantitative success, making *Dictyostelium discoideum* arguably the most modelled and best understood system in the literature of developmental biology. We do not wish to become too distracted by this model system when our focus is ultimately on multicellular organisms, so we do not here present the formulation of any of the models, rather aiming merely to give a flavour of the dynamical analyses that have been performed. We do however present the formulation of a minimal model of slime mould aggregation [185] later, in section 5.1.2, in order to set the scene for applications of the chemotactic mechanism to be discussed there.

Chemotaxis in Slime Mould Aggregation The seminal model in the study of slime moulds is the model of Keller and Segel concerning the chemotactic process leading to aggregation [185]. Assuming only the participation of random motion and chemotaxis in the aggregating movements, constant production and linear degradation of cAMP, passive diffusion of cAMP over the aggregation field, and no cell division or death in the aggregation process, a system of two partial differential equations depending on five parameters was formulated (see equations (5.4)–(5.5)). Linear stability analysis of this model reveals the possibility of instability with respect to spatial perturbations; this is interpreted as the aggregation of the initially homogeneous cell distribution to form inhomogeneous spatial structure. The position of the aggregation centres is essentially randomly determined, but once some local differences are formed — driven by internal mechanisms, some arbitrarily chosen 'pacemaker' cells begin secreting cAMP — the chemotactic mechanism is sufficient to amplify the spatial perturbation and generate structure. The formulation of this model will be briefly discussed in section 5.1.2, in the context of more general chemotactic mechanisms for pattern formation; for a lucid discussion, see also [87].

More Recent Models for Slime Mould Development The Keller-Segel model has the status more of an illustrative example, demonstrating that instability due to chemotaxis, compatible with the formation of spatial patterns in aggregation, is feasible. More recent modelling has attempted to gain a detailed, more realistic dynamical understanding of the experimentally observed biochemical processes. In particular, there is a fairly comprehensive biochemical literature on the cAMP system, as well as an adequate appreciation of cell-cell communication. Based on these experimental results, quantitatively accurate models for the secretion, activity and signalling of cAMP have been proposed.

The model for cAMP signalling of Goldbeter and Segel [122] provides an example of the detailed modelling of the participating enzymatic processes: it is based on the functional coupling of a receptor protein embedded in the cell membrane, which binds extracellular cAMP, to the enzyme adenylate cyclase, which transforms ATP to intracellular cAMP which can then be secreted to the extracellular medium and activate more receptors. The cAMP synthetic process is thus self-reinforcing, or autocatalytic, leading to destabilization. This mechanism is discussed by

Nicolis and Prigogine [277], as well as by Babloyantz [10], who also presents a brief mathematical formulation. Peacocke also discusses a kinetic model for *Dictyostelium*, by comparison with similar mechanisms that have been proposed for other systems, in particular for glycolytic oscillations [303]. A more recent and accurate model has been proposed by Martiel and Goldbeter [223] to describe cAMP signal-relaying in well-stirred suspensions of *Dictyostelium* amoebae. This model has been extended by Tyson and coworkers [356] to incorporate spatial effects, and it was shown that the combination of modelling based on detailed kinetic rate laws, and analysis depending on the mathematical properties of nonlinear systems analogous to reaction-diffusion equations, is able to account naturally and with quantitative accuracy for observed travelling wave geometries such as the formation of concentric circles and spiral waves.

Other aspects of *Dictyostelium* development have also been studied: for instance, the regulation of the prespore-prestalk ratio has been investigated [227]; and a comprehensive model for the locomotion of the slug has been proposed [282]. The extent of theoretical interest in this system is demonstrated by the high proportion of contributions on *Dictyostelium* presented at a mid-1980s conference on morphogenesis (Kyoto, 1985) [342]. A feature of this work has been the constant interplay between experiment and theory, with increasingly detailed understanding of the biochemistry enabling more accurate kinetic modelling, and mathematical analyses of the resulting dynamical system providing predictions that could again be tested against experiment. The outcome of this intensive interdisciplinary attention has been a set of quantitatively accurate, but complex, models that incorporate a multitude of relevant factors, and the challenge now is to isolate those terms that capture the essential features of the observed processes (as has been done quite successfully for the BZ reaction, where the most significant factors have been included in the simplified but accurate Field-Noyes model [100] — see appendix B) [66].

The slime mould *Dictyostelium discoideum* thus provides an excellent biological example of developmental self-organization, in which the interaction of theory and experiment has enabled significant progress in understanding to be made. It is an aim of this thesis to demonstrate that such an interdisciplinary interaction may also be fruitful in the understanding of self-organizing phenomena in the development of significantly more complex higher organisms. As we shall see, at present little detailed modelling can be hoped for, and we may often obtain little more than qualitative, heuristic predictions. Nonetheless, significant progress in discerning a basis for self-organizing mechanisms in biological development has already been achieved, as will become apparent in the following chapters.

Chapter 4

Developmental Pattern Formation — A Chemical Basis

The necessary preparations have been concluded, and we may continue with the object of our study, *Models of Self-Organization in Biological Development*: In chapter 2 we were able to obtain an overview of key features of biological development. Physical and mathematical aspects of self-organization, with particular reference to the paradigmatic reaction-diffusion systems and the chemical Belousov-Zhabotinskii reaction, were then presented in chapter 3, and appendices A and B. Now that these necessary biological, physical and mathematical preliminaries have been discussed, the stage is set for the integration of this diverse range of concepts into a *unified view of development*: the remainder of this thesis will focus on models applicable to biological development, with particular reference to self-organization.

The three main concepts applicable to the generation of biological structure and functionality are *cell differentiation*, *pattern formation* and *morphogenesis*, as discussed in chapter 2. For the purposes of our study, we shall largely neglect cell differentiation, presuming it to occur autonomously and beyond the scope of our deliberations, although differentiation may be a consequence or manifestation of the pattern-forming or morphogenetic mechanisms that will be our major concern. As already alluded to, there are two fundamentally distinct approaches, which treat pattern formation either as a distinct process occurring prior to morphogenesis, or alternatively, as simultaneous with morphogenesis. These two approaches constitute different phenomenological interpretations of the pattern formation and morphogenesis process, but are most probably also distinct mechanisms employed by the embryo at different points in its development.

In this chapter, we will investigate self-organization in pattern formation under the assumption that the patterning process is *distinct* from any consequent cytodifferentiation or form changes that may result from the pattern formation. In the next chapter, we explore some consequences of the possibility that self-organization in development may occur through the *continuous interaction* of chemical and mechanical processes to generate pattern and form, with the intimate involvement of cells in the patterning process. We emphasize again that while these two approaches may appear mutually exclusive for the coherent understanding of the mechanism at work in any *particular* aspect of development, the evidence points to the involvement of variations of each scheme at different stages in the generation of structure.

4.1 Overview: Positional Information and Prepattern Mechanisms

The view that pattern formation is a process to be considered in isolation from accompanying changes in cell shape, position, characteristics or differentiation state has generally been termed the **chemical prepattern** viewpoint (see for example [288]); although we shall later consider this a slight misnomer and interpret the concept of a prepattern in a more restricted sense. Essentially, the existence of hypothetical chemical substances, called **morphogens** [354], is postulated, which by some means to be elucidated establish spatial variations in their concentrations. Once such a chemical pattern is laid down, it is 'read out' by the cells, which respond according to the local morphogen concentration, following a 'program' depending on their genetic information and previous developmental history (although see section 6.2.3 for the limitations of the concept of a developmental 'program'). Thus this approach separates the process of structure formation and differentiation in development into two distinct stages:

1. Creation of the underlying (chemical) pattern, and
2. Cellular response to the local chemical concentration.

In this view, cellular differentiation and morphogenesis are simply slave processes, that are fully determined once the chemical pattern is established; thus models and investigations of development based on such an approach focus on the problem of how the chemical pattern is laid down.

The concept of **positional information** is intimately connected to such an approach; although as we shall see, here also there is some confusion about the exact meaning to be ascribed to this term. In its most general form, positional information is the expression of the powerful idea that *the fate of a cell within a developing organism is largely dependent on its position.* This concept has long been implicit in embryological theory and experimentation, for example in the concept of a morphogenetic field proposed by Driesch, and the gradient theories of Child (see [10, p.293] and [376]), but in more modern times, the concept is associated with the work of Lewis Wolpert [375], although in a more limited context, as we shall see.

The Ubiquity of Position-Dependent Interactions

There are few alternatives to positional information in its widest form, the idea that cells receive information of some sort that is *position-dependent*, and react accordingly by changing their state in some way. In the classification of Held [159] (originally introduced by Steinberg and Poole [332]), the two other, and apparently considerably less important, possibilities, are that the state of a cell causes its position (for example by cell rearrangement based on differential adhesion; the final position of a cell is determined by its ability to adhere to some target cell), or that both position and state depend on some third, independent factor — essentially that the lineage, or developmental history of unequal cell divisions and cytoplasmic determination, influences both cellular position and differentiation in mosaic development. However, even these two possibilities may incorporate some overt position dependence: for example, an organism such as the nematode *Caenorhabditis elegans*, which under normal developmental conditions undergoes a

fully determinate pattern of cell divisions and has an invariate lineage for each somatic cell, shows evidence of inductive interactions due to neighbouring cells when its development is disturbed (see section 2.2.1).

The concept that the environment of a cell plays a fundamental role in its development is thus a very general one, and incorporates a wide variety of possible interactions that may provide positional information in this sense. For example, inductive interactions, in which cells receive signals from adjacent 'inducers' which influence their state of differentiation and subsequent development (the classical example is the amphibian 'organizer' discovered by Spemann and Mangold — see section 2.2.2), form positional signals; so do signals about the local stress or curvature of the tissue of which the cell forms a part, adhesive interactions with adjacent cells, chemical signals transferred by contact or through cell junctions, local electric fields, mechanical strain fields, or any number of other possibilities. Using this interpretation, positional information also encompasses the signals that lead to morphogenetic movements, causing cells to participate in patterning and morphogenesis and to change their neighbours. The basic characteristic of positional information in this sense is that *the cell under study would receive a different positional signal if it were elsewhere in the embryo*, and thus its subsequent development would be different. Of particular interest to us in this chapter, positional information in this sense can also incorporate chemical concentration fields, such that cells 'read off' the local concentration of some regulatory molecule, or morphogen, and respond accordingly through differentiation and morphogenesis.

Topobiology The emphasis on the primacy in development of 'place-dependent interactions' has been made especially by Edelman, who has coined a term, *topobiology*, to describe the dynamic, interactive process of interaction between cells and their environment (see for example [84]). He has, however, limited his focus particularly to the local cell-cell and cell-substrate interactions, mediated by the cell adhesion molecules (CAMs) and substrate adhesion molecules (SAMs) as the main 'morphoregulators' or determining factors of the primary morphogenetic process [80, 81, 110] (see section 2.1.2). It will become increasingly apparent throughout this thesis that although the molecules responsible for local adhesion are clearly highly significant to development, this view of cell fate being determined solely, or predominantly, by local interactions with neighbouring cells, membranes and extracellular matrix, appears to be too narrow, in view of the variety of other interactions that may contribute to morphogenesis; furthermore, such 'topobiology' goes no way towards accounting for pattern formation and its control in first place, and thus, in spite of its suggestive-sounding name, is no major contribution to the issue of the specification of positional information that we are concerned with here.

Positional Signals — Instruction or Selection? We must note that no matter what the nature of the positional signal that the cell receives, the response of the cell to the signal is limited by the *competence* of the cell. At each stage in development, a pluripotent cell has a, generally fairly limited, range of further developmental options open to it; these are based on its previous developmental history, which determines its current state of gene expression, cytoplasmic composition, structural properties and geometry, and those other characteristics summarized in the epithet of cell 'state'; of course, the hereditary 'information' contained in the genome imposes an ultimate constraint on the developmental pathways that are available. The positional signal can only provide a basis for the choice of one of these options, but cannot

introduce new information, in the sense of 'telling the cell something it does not already know' [378]. Thus signals must be essentially *selective*, rather than instructive, which emphasizes the primacy of the cell's internal programme — the complexity of cellular behaviour, in terms of multiplicity of resultant states, lies ultimately in the cell's capacity to respond rather than in the signals.

We have here considered 'positional information' in its widest sense, of *position-dependent* information; the general terms introduced above have more restricted, and hence possibly more useful, meanings, however: The concept of autonomous pattern formation, independent of cellular responses in differentiation and morphogenesis, is generally considered to embody two distinct possibilities by which cells respond to preexisting chemical concentration patterns. The overlap of terminology that will be apparent here reflects some confusion in the literature, but the definitions to be introduced below will be retained throughout the remainder of this discussion: the two possibilities are known as *positional information* and *prepattern formation.*

Positional Information

The more restricted meaning of the concept of **positional information**, and the sense in which it was originally introduced by Wolpert [375], refers not so generally to information *dependent on* the position of the cell, but more specifically to information *about* the cell's position. In particular, it is assumed that cells in a developing system can 'know their place', by having their position specified relative to the other cells or with respect to one or more fixed points of the system. This implies the presence of some form of *coordinate system*, and a means for transmitting information about that coordinate system to the cells. Furthermore, a polarity is required, to define a direction in which the positional information is specified or measured. The means by which such a coordinate system is established is then generally the major object of study of such a model.

The latent assumption is that cells are able somehow to 'read off' and interpret the positional information according to their "genetic constitution and developmental history" [377, p.441], responding in some appropriate way, such as by differentiation. This is thus fundamentally a *two-step process*, comprising firstly the specification of some form of, generally molecular, 'address labels', and secondly the cellular response. The foremost requirement for such a response is that the cells acquire **positional value**, the long-term memory of position, which must be distinguished from any positional signal by which it is specified [377]: Any such signals are necessarily transient, but the cells must retain the knowledge of their position with respect to the overall organism. Positional value is successively specified, locating the cell with ever finer detail in the adult organism, and thus constitutes a form of cell determination — early spatial signals specify crude global coordinates, and subsequent more local signals supply additional precise details of the address relative to local 'landmarks'. Thus each cell is provided with a unique address (up to the precision with which the positional information can be read — see section 4.2.3) thus rendering cells, even those of overtly the same differentiation class, nonequivalent.

One feature of positional information is that it provides only coordinates; there is no direct correspondence between the 'map' of positional values that the cells obtain and the final pattern. The generation of the *details* of the pattern lies in the way that the cells *interpret* the positional information — we essentially require complex responses to simple information, with

the responses arising from the potentialities of the cells' internal developmental programmes. As a consequence, it was originally postulated [375] that the same positional field might be used for very different anatomical patterns, as the resultant pattern follows from the cell competencies, not the coordinates. This led to the hope that universal signalling mechanisms or coordinate systems could be found which would provide a basis for positional information and consequent pattern formation in a wide range of developing systems and organisms. This hope has however not been fulfilled; among the negative indicators against a universal morphogenetic signalling system is the very different chemical nature of the molecular species that have been shown to have graded concentrations and hence probably to participate in some form of signalling [326] (see section 4.2.4).

Chemical Prepatterns

The most popular and widely accepted models for the specification of positional information, as we shall see, involve graded concentration patterns of some chemical, and thus fall into a 'chemical prepattern' conceptual framework in the wide sense considered above. The narrower, and hence more meaningful, sense of the term 'prepattern' is by contrast with the above concept of positional information: In positional information, essentially simple information, containing only the position of the cell with respect to some coordinate system, triggers a complex interpretive response from cells; there is no overt correspondence between the signalling system and the resultant pattern. A **chemical prepattern**, on the other hand, is one in which the final pattern is essentially established in the concentration pattern of some putative chemical, or *morphogen*. It is assumed that cells respond in a certain way, for example by differentiation, if the morphogen concentration is, say, above some threshold value.

In this case the final pattern is contained essentially in the underlying chemical prepattern, so that its complexity depends on the pattern-forming potential of the mechanism responsible for the prepattern; we may speak of a morphogen pattern homologous or 'isomorphic' [264] to the biological pattern. The cellular mechanism required to interpret the prepattern may be a simple response to a complex prepattern, which contains sufficient spatial information to account for the features of the biological pattern — cells can be "stupid" [159]; the feasibility of the prepattern concept relies on the existence of mechanisms able to produce chemical prepatterns of the requisite complexity, that is on the pattern-forming properties of prepattern mechanisms. Again, the patterning mechanisms are also potentially universal, although in view of our experience of the range and complexity of developmental mechanisms, such universality is probably unlikely. The fundamental distinction between positional information and prepattern mechanisms for producing complex pattern thus lies in the origin of the complexity: in the first case, we have a complex cell response to simple coordinate information, while the second case is based on complex chemical prepatterns requiring only a simple cell response.

Required Ranges of Validity We will consider both these possibilities, positional information and prepattern formation, for the autonomous creation of spatial pattern in some depth, but first we note that neither of these mechanisms, nor any other pattern-forming mechanism, needs to be able to account for the full complexity of biological pattern. Pattern formation is a sequential process, with spatial differences first being laid down crudely, and then at successively finer levels of detail; ultimately biological shapes are finely sculpted and refined through local cell-cell

interactions. There is a hierarchy of patterning mechanisms involved in the formation of any one organ; the same mechanism could be employed repeatedly, or different patterning methods could succeed each other. Positional information and prepattern mechanisms are not mutually exclusive, nor do they exclude other more interactive mechanisms such as the cell motions or mechanical forces we will discuss in the next chapter; different strategies could be employed at different stages, in consonance with experimental investigation of developing systems which usually points to a variety of patterning techniques.

A consequence of the processes of random mutation and natural selection during evolution is that the mechanism that was 'chosen' to generate a particular pattern is not necessarily the most obvious, or simplest, or most efficient, or the one which best fits into some overarching conceptual scheme about how development 'should be' controlled; but rather the one that 'happened to be available' and do the job. Such considerations are intended to counteract the absolutism recurrent in the literature, where a patterning mechanism is frequently required not merely to be able to account for *some* pattern, but to be a reasonable explanation for *all* pattern. What is too often sought is not a working concept, but a general principle, which overlooks the *contingent* nature of biological organisms arising from evolutionary processes.

As an example of such misconceptions, we find an otherwise sound discussion concluded by a question mark being placed behind positional information mechanisms in general because, even taking into account hierarchies of nested gradients, "the level of detail in most anatomical patterns is orders of magnitude greater than ...a striped *Drosophila* embryo ... To appreciate the dilemma, consider that each of the million-or-so hairs in your skin would have to possess a unique 'area code' and decipher its code by looking it up in the genetic equivalent of an enormous 'area-code/differentiated-state' directory" [159, p.13] (here a prepattern mechanism, for instance, might do perfectly well, especially as no high accuracy and reproducibility of patterning is required).

For the opposite extreme, consider the following recent extravagant claim for the universal pattern-forming potentiality of reaction-diffusion systems, which fall in the class of prepattern mechanisms — these have "the advantage of being able to reproduce an impressively large variety of patterns, perhaps even any pattern observed in nature" [72, pp.547–8] (compare appendix A.4); the same author, however, continues by rejecting reaction-diffusion systems on account of their failure to regulate *ab initio* (compare section 4.3.5), without being prepared to consider them even for situations where regulation is not required. The fallacy in both these quotes is that no mechanism can be, or need be, expected to fulfil the requirements of every developmental pattern formation situation; according to our present state of knowledge about pattern formation, there are in all likelihood usually several mechanisms at work simultaneously or sequentially, so that exclusive or categorical statements are fundamentally out of place.

With this cautionary comment in mind, we may proceed to consider some of the ramifications of the concept of positional information.

4.2 Positional Information

The general concept of positional information embodies, as we have seen, a two-stage process:

1. Cells receive information about their positions with respect to some coordinate system, thus potentially acquiring knowledge about their relative place within the developing embryo.

2. Cells then *interpret* this positional information based on their internal competencies, thus obtaining a *positional value* which influences or even determines their subsequent development.

The study of positional information tends to be most concerned with the first stage, the mechanism for the generation of the field of unique positional information. An interpretation in terms of the linearly graded distribution of some morphogen has generally been considered natural and most popular, although some non-gradient mechanisms will also be considered (section 4.2.5). One need not necessarily even be restricted to one-dimensional or rectagular coordinate systems, and a polar coordinate model has been applied extremely fruitfully to particular problems, especially related to regulation (section 4.2.6). To balance our study, we also briefly consider stage 2, the interpretation of positional information (section 4.2.3). Note that many of the approaches considered in this section are *not* self-organizing, but are included for completeness and comparison with prepattern mechanisms and the self-organizing mechanisms for gradient formation (section 4.2.2).

4.2.1 Cartesian Coordinate Systems and Gradients

The most natural means of specifying position clearly corresponds to a Cartesian coordinate system, which defines the position of the cell along an axis relative to some arbitrarily chosen origin at one end. By this means, complete positional information within a developing system may be transmitted by coordinates along just three orthogonal axes, which would imply a very 'simple' mechanism for pattern formation — except of course that the interpretation of the information to produce appropriate patterning is highly nontrivial. We consider only one space dimension for simplicity; clearly analogous means may be used to specify position in three mutually perpendicular directions.

The usual mechanism proposed for the transmission of positional information along a single axis is the action of an extracellular chemical signal, denoted an inducing factor or morphogen, which is expressed in a monotonic concentration gradient. We require monotonicity, that is the absence of maxima or minima, in order to establish a distinction between this mechanism for the specification of positional information and a prepattern mechanism, which contains localized peaks and troughs in concentrations.

Gradients in some (usually unspecified) chemical, or in some other cell property such as 'metabolism', have long been proposed to 'explain' the gradations in cell and tissue properties observed in development, but it is only recently that gradients in molecular species have been experimentally observed (see section 4.2.4), which has greatly boosted the plausibility of the involvement of gradients in pattern formation. "Morphogen gradients used to be regarded by

molecular biologists as a kind of mystical pseudoexplanation and a distraction from the serious business of molecular-genetic analysis, but this prejudice has subsided in recent years as the gradients themselves have begun to be discovered" [327, p.43].

Diffusion

The most usual and simplest form of the Wolpert positional information concept is to assume that the concentration gradient is produced by some *diffusible molecule*, which is emitted by a localized source whose existence and position are postulated or taken as experimental fact; no demand is made on the theory to account for the origin of the sources. Thus an initial inhomogeneity, constituting a definite polarity in the field, is required, whose presence and location are communicated throughout the field by the diffusing morphogen.

Diffusion as a Random Walk Process The physical basis of diffusion is a random walk process: the random thermal motion of molecules in all directions will ultimately result in a net flux of molecules from a region of high concentration to low concentration of a substance; a flux which, to first approximation, is proportional to the local concentration gradient. This process is modelled by Fick's law, such that the diffusive flux J, as a function of the concentration c, is given by

$$J = -D\nabla c, \tag{4.1}$$

where the minus sign indicates that the flux is *down* the concentration gradient. Models involving diffusion (more generally, the setting up of gradients) are usually one-dimensional for ease of computation, referring either to diffusion along a line of cells or across a sheet of cells in which any sources and sinks are arranged as transverse rows; in this case, the above gradient operator becomes dc/dx. D is the diffusivity, which depends both on the characteristics of the molecule in question and the viscosity of the medium, and is a direct measure of the speed of spreading. The values of D for various substances in cytoplasm have been measured (see [67]): for small molecules, values are in the range 1×10^{-6} to 5×10^{-6} $\mathrm{cm^2s^{-1}}$, whereas values for the considerably more bulky proteins are much lower, of the order of 0.3×10^{-8} to 1×10^{-8} $\mathrm{cm^2s^{-1}}$. D could also be treated as a variable which depends on the state of the cells, or on the space coordinate, especially if transport mechanisms involve passage through gap junctions or carrier molecules [327].

The dynamical effect of diffusion on the local concentration of a substance is obtained by applying mass balance concepts, noting that the change in concentration in some small volume is due to the difference between the fluxes in and out of the volume. When these effects are considered in the limit of small volumes (to obtain the local behaviour), we obtain the prototype parabolic partial differential equation (one with a first derivative in time and a second space derivative), the diffusion equation, which has been implicit in our earlier discussions of reaction-diffusion equations (see section 3.2.2, and appendix A):

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{4.2}$$

(in one dimension).

An immediate consequence of the diffusion equation is that, in the absence of conflicting boundary conditions, the steady solution is c = constant, so that concentration inhomogeneities are destroyed. This is the motivation for the description of diffusion as a 'smoothing mechanism', and the reason why the discovery that diffusion in the presence of reactions could be a destabilizing effect, resulting in spatial inhomogeneities, was so counterintuitive and unexpected [354] (see section 3.2.2).

In order to produce some concentration variation, therefore, it is necessary to have *nonuniform* boundary conditions; that is, the substance under consideration is being produced in some places (*sources*) and removed in others (*sinks*). In this case, the distribution of material will tend to an inhomogeneous steady state, in which $dc/dt = 0$ everywhere, and there is a continuous flow of material from the sources to the sinks.

Diffusion and Symmetry-Breaking As the source must already be distinguished from its surroundings, there is preexisting inhomogeneity, so that the diffusion process does not lead to the creation of any new or enhanced structure as such. Moreover, the threshold responses of cells based on the concentration levels cannot be said to involve the breaking of any symmetry or creation of any novel structure, as opposed to exploring the latent potential of the gradient-mediated polarity that is already there. Hence such a diffusion mechanism cannot be classed as a self-organizing mechanism *per se*. Nevertheless, we shall consider diffusion in some detail, in particular for comparison with reaction-diffusion mechanisms, which *are* self-organizing and may also form gradients (section 4.2.2).

It is useful to see clearly that there are also non-self-organizing models, mathematically formulated and amenable to quantitative analyses, which may make similar predictions to those of self-organizing models, based on considerably different assumptions, especially about the heterogeneity of the initial conditions. The study of gradients occupies a special place in the modelling of pattern-forming mechanisms, both historically, and in the light of recent experimental demonstrations of the existence of concentration gradients at crucial stages in the development of some organisms (see section 4.2.4) — frequently, in fact, source regions have been identified. It is thus appropriate to indicate the major explanatory paradigms proposed for gradient formation, even if they are not all based on fundamental symmetry-breaking.

Source-sink Model for Diffusion

The simplest and most obvious model for diffusion to consider, is one in which the diffusible chemical 'morphogen' is produced at one end of an array of cells, the 'source', and consumed at the other end, the 'sink'. In this case, the steady state concentration profile is a linear gradient, obtained from the solution of the diffusion equation. For example, if the source at $x = 0$ produces morphogen to maintain a constant concentration of $c = c_0$, and the sink is at $x = l$, holding the concentration there to zero, then the resultant gradient obtained from the steady state solution of the diffusion equation (4.2) is

$$c = c_0(1 - \frac{x}{l}). \tag{4.3}$$

Simple diffusion as a process for setting up gradients of chemical concentrations, was long viewed with suspicion and mistrust; not because of the above-mentioned steady state properties,

which gave the required gradients, but because of the *time* that would be required to establish the gradient from just, say, a source at one end, and an initially uniform concentration throughout the line of cells. The reason is that, since diffusion is a random walk process, it can be shown that the time taken to establish a concentration profile over a domain of length l grows as l^2; thus diffusion is very rapid over short distances, but slow over long distances.

It has been estimated [375], in support of positional information mechanisms in general, that embryonic patterns are generally laid down over fields of the order of a millimetre, or 100 cell diameters or less; and that this pattern is then retained and refined through subsequent growth, and the laying down of more detailed patterns. Furthermore, a time of the order of a few hours appears to be an average time scale over which patterns are produced. Any reasonable pattern-forming mechanism should be able to satisfy the above time constraints in addition to generating plausible patterns. The initial perception was that simple diffusion was just too slow to be plausible.

Crick — Estimate of Diffusional Times This concern was dispelled through an order-of-magnitude calculation by Crick [67], who set out to show — with considerable success, judged by the impact of the work — that embryonic concentration gradients established by diffusion between a source and a sink were feasible. A range of calculations under different initial and boundary conditions was performed [244], to estimate the time needed to set up a gradient; the results in general could be expressed in the form

$$t \approx A\frac{n^2 l^2}{D}, \tag{4.4}$$

where t is the time needed to obtain a satisfactory approximation to the final gradient, n the number of cells between source and sink, l the length of each cell in cm, D an effective diffusion coefficient, which might incorporate the effects of facilitated diffusion, and A a numerical constant, in the range of about 0.1 to 0.5, whose value depends on the way the gradient is developed. Crick showed that for small molecules, with $D \sim 10^{-6}$ cm^{2}s^{-1}, a few hours was sufficient time to establish gradients over fields of several tens of cells, in accordance with Wolpert's estimate [375] of the distance over which fields are established. Crick concluded that the times and distances involved in patterning in many embryological situations are broadly compatible with diffusion-based mechanisms [67, 68], a result which firmly established the feasibility of gradient explanations, mostly mediated by diffusion, for the laying down of positional information.

Disadvantages of the Source-Sink Model Crick's calculations are today of considerable historical interest, as they had a marked impact in reawakening interest in diffusion and gradients. They are however no longer considered seriously as a mechanism for pattern formation; the problem is still with the times involved: A glance at the diffusivities listed above indicates that for larger molecules such as proteins (which, as we shall see below, have been experimentally implicated in gradients — see section 4.2.4), diffusion constants are of the order of 10^{-8} cm^{2}s^{-1}, in which case Crick's order-of-magnitude calculations immediately indicate that the time to set up an approximation to the gradient increases hundredfold and is hence unrealistic. Furthermore, the source-sink model requires there to be *two* specialized regions to establish positional information for the tissue in between. These drawbacks have led to faster alternative formulations of gradient formation models, based both on simple diffusion and on other mechanisms.

Localized Source — Dispersed Sink

The most popular diffusion-based mechanism for establishing a monotonic concentration profile postulates a source of the substance, with fixed concentration c_0, and assumes that the diffusible morphogen is broken down *throughout* the field, at a rate which is to first order proportional to the local concentration, so that the entire responding field acts as a sink to the morphogen; this model may be referred to as the 'localized source and dispersed sink', or LSDS, model [327]. The existence of a specialized sink at the far end of the field is avoided by merely assuming zero flux boundary conditions at $x = l$, that is, the end of the field acts as a barrier for the morphogen. The formulation of this model is thus

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - kc, \tag{4.5}$$

where k is the rate of linear degradation of the morphogen, with boundary conditions $c = c_0$ at $x = 0$, $dc/dx = 0$ at $x = l$. This model gives an exponential concentration profile, with a steady state solution of

$$\frac{c}{c_0} = e^{-\alpha x} + \beta \sinh \alpha x, \tag{4.6}$$

where $\alpha^2 = k/D$ and $\beta = 2/(1 + e^{2\alpha l})$.

The calculation of transients, which is a complex exercise analytically but straightforward if the equations are solved numerically on a computer, indicates that an acceptable approach to the steady state can be achieved in one hour, on a field of about 1 mm in length, for a diffusion constant of $D \sim 10^{-7}$ $\mathrm{cm^2 s^{-1}}$; this corresponds well with the experimentally observed and estimated ranges. Thus the LSDS model appears to provide a feasible mechanism for gradient formation, based on diffusion.

Regulative Properties of the LSDS Model The analysis of proposed patterning models such as gradient models must consider their *regulative properties*, that is, whether the predicted behaviour in the face of experimental manipulations accords with observations. We shall not consider such studies in detail — see for example [327, 375], as well as [159] and references therein — but note that a model such as the LSDS model immediately accounts for the special properties of organizer regions (see section 2.2.2), by identifying them with sources: for example, if the source region is removed, then the steady state concentration everywhere falls to zero, and no structure is formed, whereas the transplantation of the source to the other end of the field results in the structures forming in the correct sequence, but with an overall reversal of polarity; if a second source is grafted to the other end, then a U-shaped gradient pattern will result, in agreement with the experimentally observed duplication of structures arranged with opposite polarity around a central plane of mirror symmetry. Further important features, such as defect regulation, corresponding to the regeneration of the gradient if a part of the field different from the source is removed, and the coordinated determination of structures at different distances from the organizer owing to a threshold response mechanism (discussed below in section 4.2.3) also arise naturally out of such a gradient model [327]. In fact, the original concept of positional information [375] developed out of the need for a unified account of regulative behaviour [159].

Gradient models and their analysis, as reported in the literature, provide a good example of the constant interplay between theoretical predictions and the use of focussed experimentation

to test these predictions, especially with respect to the predicted and observed behaviour in the face of perturbations and disturbances of normal development; if necessary, such comparisons stimulate the development of an improved theory. The successes of the experimental search for gradients will be reported on below (section 4.2.4).

4.2.2 Self-Organizing Gradients — Reaction-Diffusion Models

The simple diffusion of a morphogen in response to a preexisting polarity, assuming the *a priori* presence of a source and one or distributed sinks, is probably the most obvious way to specify one-dimensional positional information by the creation of a gradient, but it is by no means the only way. From a philosophical viewpoint, a conceptual framework for pattern initiation indeed appears slightly unsatisfactory if it is forced to assume asymmetries from the outset, although in a given developmental situation such initial polarities and sources may of course exist. For theoretical purposes it is frequently superfluous to postulate initial inhomogeneities beyond the scope of the model, as we saw in the previous chapter; there exist self-organizing mechanisms that may account for the spontaneous generation of pattern and polarity through the instability of a homogeneous state.

Reaction-Diffusion Equations and Spontaneous Gradient Formation

The paradigmatic example of self-organizing dynamics is provided by reaction-diffusion systems, comprising two or more chemical species interacting through appropriate nonlinear reaction kinetics generally containing some form of auto- or cross-catalysis and inhibition, which are able to diffuse within the domain at different rates (see the extensive discussions in section 3.2 and appendix A). Our previous discussion was presented as a study of chemical kinetics with application to the model Brusselator reaction scheme [310] (section 3.2.3) and the Belousov-Zhabotinskii reaction (appendix B), and without any explicit reference to biological pattern formation.

It should however be apparent that, given that biological processes are based on biochemical (metabolic) reaction pathways, reactions and diffusion are ubiquitous in development. There is thus no conceptual hindrance to the application of such motifs arising from chemical dynamics studies to biological pattern formation, with at least one of the chemicals involved in the putative reaction-diffusion scheme acting as the *morphogen*, or form-producing molecular species. Indeed, the original motivation for the study of reaction-diffusion equations [354], and much of the impetus for their subsequent study, has been to discover a basis for biological pattern formation and morphogenesis.

The potential for the generation of inhomogeneous spatial 'dissipative' [276] structures through a reaction-diffusion mechanism has already been demonstrated. In particular, it has been shown that, at least immediately beyond the instability or bifurcation point, the patterns predicted by linear theory correspond to the eigenfunctions of the Laplacian operator for the domain and boundary conditions of interest (see appendix A.3). For a reaction-diffusion system operating in one space dimension, with zero flux boundary conditions — that is, the pattern is formed autonomously, with no communication with the zone outside the domain — this leads to cosinusoidal solution patterns, with an integral number of nodes in the region and with antinodes located at the boundaries. The simplest case of this arises when the domain length is just

above the critical length needed to accommodate any inhomogeneity at all; here exactly one node, $n = 1$, can fit into the domain, so that a half-wave pattern is formed, with a crest at one end and a trough at the other — in short, a *gradient*.

The generation of a chemical gradient by a reaction-diffusion system provides a means of retaining the positional information concept and establishing a coordinate system, without the need to invoke the preexistence of localized sources. The amplification of the linearly unstable solutions needs only small random space-dependent fluctuations from the homogeneous steady state. As a half-wave pattern is generally the first spatial pattern to arise from initial homogeneity as a developmental domain grows in size, it may not even be necessary to postulate the variation in any parameter other than domain size to 'switch on' the reaction-diffusion system and trigger the instability. A basis to account for symmetry-breaking, the formation of polarity *de novo*, in biology is thus obtained; this is a fundamental characteristic and contribution of self-organization.

Motivation for the Study of Symmetry-Breaking Mechanisms The question of the extent to which the study of such symmetry-breaking features are strictly pertinent to any developing system will concern us in more detail later (see [112]), but it suffices for now to note that there are, at least, reasons for thinking that in some cases certain regional differences in very early development are set up in response to no stimuli beyond very minor environmental perturbations. One can never be totally sure that an ostensibly symmetry-breaking process is not merely the manifestation of some cryptic preexisting asymmetry. Nevertheless, in examples such as the orientation of the dorso-ventral axis in the amphibian egg, or of the cranio-caudal axis in the isolated anterior half of an avian blastoderm, or the position of the blastocoelic cavity in the mouse embryo, there is no obvious initially determined polarity, and a positional information mechanism based on overt sources and sinks certainly seems inappropriate [327]. For such situations, we turn to self-organizing mechanisms, within which spontaneous symmetry-breaking and the generation of random orientations are fully explicable as the amplification of microscopic perturbations into macroscopic inhomogeneities, in the intimate interaction between deterministic dynamics and fluctuations.

We shall return to the early history of the applications of reaction-diffusion models in pattern formation (see section 4.3), but note here that probably the earliest models for spontaneous gradient generation in multicellular organisms are those due to Gierer and Meinhardt; their schemes have certainly had considerable impact, drawing the attention of biologists to the potential, counter-intuitive pattern-forming properties of such kinetic schemes, especially with the aid of computer simulations of the nonlinear reaction-diffusion equations with a resemblance to many patterns formed in nature [87].

The Gierer-Meinhardt Activator-Inhibitor Model

The work of Gierer and Meinhardt, especially their original paper [114], contains a variety of different schemes with different pattern-forming properties (see the reviews in [112, 229, 233]). The motivation for the study was by analogy with lateral inhibition in other contexts, and the proposed mechanisms involved an activating molecule interacting either with an inhibitor, or with some other substance whose concentration was somehow depleted. On this basis, a variety

of self-organizing models has been proposed, both for gradient formation (to be discussed here) and for the generation of more complex patterns (see section 4.3.1).

The fundamental conceptual contribution of their work comprised the recognition that the interaction between short range activation and long range inhibition is sufficient to account for the formation of morphogenetic patterns — see the heuristic description in section 3.2.2 [112, 303]. To support their contention that such schemes were plausible, different kinetic realisations of lateral inhibition mechanisms were presented, with different activator-inhibitor interactions and sources, interconversion between the two chemical species, saturation of activator production, and so on.

One of their activator-inhibitor schemes has been especially popular, and is the one frequently referred to in the literature as the 'Gierer-Meinhardt model' (see for example [249, 324]). In their original notation, denoting the activator concentration by a and that of the inhibitor by h, the equations are (in one space dimension) [114, equations (15a) and (15b)]

$$\frac{\partial a}{\partial t} = \rho_0\rho + c\rho\frac{a^2}{h} - \mu a + D_a\frac{\partial^2 a}{\partial x^2}, \tag{4.7}$$

$$\frac{\partial h}{\partial t} = c'\rho' a^2 - \nu h + D_h\frac{\partial^2 h}{\partial x^2}. \tag{4.8}$$

Here the activator and inhibitor are both assumed to decay, at constant degradation rates μ and ν, respectively; ρ and ρ' are the source concentrations for activator and inhibitor, and ρ_0 is the basic production of activator, such that for $\rho_0 \neq 0$, the first term in (4.7) refers to a steady supply of activator due to some external source; furthermore, D_a and D_h are the diffusion coefficients of activator and inhibitor, respectively. The crucial terms in the equation formulation are those which model the interaction between the two chemical species: The autocatalytic production of the activator is modelled by the term $c\rho a^2/h$, and the inhibitory effect is contained in the denominator, $1/h$, counteracting the autocatalytic production rates. The activator also stimulates the inhibitor production, at a rate $c'\rho' a^2$. This formulation (4.7)–(4.8) satisfies the general two-variable reaction-diffusion scheme, and may by a suitable nondimensionalization be converted into the form, for instance, of equations (A.19)–(A.20).

A Molecular Basis for the Gierer-Meinhardt Equations The model justification in the original papers by Gierer and Meinhardt [114, 234] was only heuristic, not derived from the kinetics of any particular reaction scheme. Babloyantz and Hiernaux [8, 13] (see also the discussion in [10]) proposed a molecular scheme capable of accounting for the onset of polarity in an initially uniform system, consisting of the interaction between activating and inhibiting morphogens, X and Y:

$$\mathrm{A} \xrightarrow{k_1} \mathrm{X} \tag{4.9}$$

$$\mathrm{C} + 2\mathrm{X} \xrightarrow{k_2} \mathrm{Y} + 2\mathrm{X} \tag{4.10}$$

$$\mathrm{X} \xrightarrow{k_3} \mathrm{F} \tag{4.11}$$

$$\mathrm{Y} \xrightarrow{k_4} \mathrm{G} \tag{4.12}$$

$$\mathrm{Y} + \mathrm{P} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} \mathrm{X} \tag{4.13}$$

$$\mathrm{B} + \mathrm{X} + \mathrm{P} \xrightarrow{k_7} \mathrm{P} + 2\mathrm{X} \tag{4.14}$$

With the exception of the fifth reaction, the inverse reactions are neglected; thus the system is automatically operating at far from thermodynamic equilibrium, satisfying an important requirement for the possibility of dissipative structures (see section 3.1.3).

If a quasi-steady state assumption is made for the substance P, and diffusion of the morphogens X and Y is taken into account, then the kinetics of the above scheme may be described by a system of two coupled reaction-diffusion equations,

$$\frac{\partial X}{\partial t} = k_1 A + \frac{k_6 k_7 B X^2}{k_5 Y} - k_3 X + D_X \frac{\partial^2 X}{\partial x^2}, \tag{4.15}$$

$$\frac{\partial Y}{\partial t} = k_2 C X^2 - k_4 Y + D_Y \frac{\partial^2 Y}{\partial x^2}. \tag{4.16}$$

To complete the formulation, appropriate boundary conditions, such as zero flux conditions, must be specified, and initial conditions, for example random perturbations, must be assumed. A comparison of equation systems (4.7)–(4.8) and (4.15)–(4.16) immediately demonstrates the mathematical equivalence of these two models, but with different interpretations of the parameters. In consequence, the stability analyses and discussions of the nature of the bifurcating solutions provided in [13] automatically carry over to the Gierer-Meinhardt equations.

Solution Properties of the Gierer-Meinhardt Equations As is evident from our previous mathematical analyses of reaction-diffusion equations, the Gierer-Meinhardt equations may show spatially inhomogeneous solutions bifurcating from the uniform steady state, provided the equation parameters lie within the 'Turing space' [249], the region within which diffusion-driven instability is possible. It is pertinent to note here that a comparative analysis of the sizes of the Turing spaces of the Gierer-Meinhardt system (4.7)–(4.8), the Thomas substrate-inhibition system (3.15)–(3.17) [188], and the simplest Schnakenberg system [317] (see section 3.2.3) has been performed [249], with the conclusion that, while the Thomas system in particular is a very robust mechanism, the Gierer-Meinhardt model has a relatively small Turing space. This means that for pattern formation to occur, the parameters must be finely tuned, which may be unrealistic in the face of inevitable environmental perturbations. What *is* apparent, however, is that the Gierer-Meinhardt equation system may be considered as a prototype for an entire class of reaction-diffusion equations with the property that, for appropriate parameter values, instability of the homogeneous equilibrium may occur.

In a growing domain, there is a critical domain size for pattern formation; for regions too small to fit in half a wave at the chemical wavelength, homogeneity remains. Beyond that size, the first inhomogeneous pattern forms, which as already emphasized, is a half-wave, or gradient. More precisely, parallel gradients form in the concentrations of both the activator and inhibitor, so that models for gradient formation such as that of Gierer and Meinhardt are frequently referred to as 'double gradient' models (see for example [327]) — this is by contrast to, for example, the Brusselator model, in which the two concentration gradients run in opposite directions (see [150]). It is assumed that at least one of the chemicals involved in the gradient formation acts as a morphogen; thus the Gierer-Meinhardt model provides a scheme for positional information, in an analogous way to the gradients resulting from source-sink diffusion or LSDS, and with analogous problems of interpretation (see section 4.2.3 below).

The Specification of Polarity It is pertinent to note that our mathematical analyses of the nature of the bifurcating solution (see appendix A.3) demonstrated a two-fold degeneracy of the solutions; that is, patterns with polarity in either of the two available directions in the one-dimensional system could form. The direction of the asymmetry after the diffusive-driven instability is determined by the dominant polarity of the perturbations which trigger the instability. Thus, if no preexisting inhomogeneities are assumed, and the structure is formed purely by the interaction of reaction and diffusion to amplify random perturbations of a uniform equilibrium, the polarity of the final gradient is intrinsically *undetermined* and could be in either of the possible directions, with equal probability.

This is all very well if we are dealing with an initial symmetry-breaking event in the early embryo, in which case a pure self-organization process may well be appropriate; but later embryonic structures, such as limbs, must form in a well-defined orientation with respect to the rest of the embryo. To guarantee this desired polarity, the symmetry-breaking mechanism must be biased in some way [112]. In their original models, Gierer and Meinhardt achieved this polarity specification by assuming a spatial dependence for the source distributions, considering especially ρ_0 to depend on x. Then a weak gradient in ρ_0 could serve to fix the resultant polarity of the system.

There has been some controversy regarding this approach [87], as there is then no longer pure symmetry-breaking and self-organization — whereas the peculiar feature of reaction-diffusion mechanisms is precisely that initial biases and nonuniformities are *not* required for structure formation. On the other hand, in a situation where the correct polarity of a pattern *vis-à-vis* the rest of the organism is crucial, there has to be some way of constraining the direction of the asymmetry, and the assumption of prior homogeneity is then, strictly speaking, invalid anyway. The peculiar self-organizing properties of reaction-diffusion systems by no means become redundant if there are initial inhomogeneities, however. In particular, unlike equilibrium diffusion processes, reaction-diffusion systems have the ability to generate stable, steep gradients from shallow source gradients, which persist in form and orientation when the source gradient is removed.

The gradients formed by reaction-diffusion systems, in particular the Gierer-Meinhardt system, have other desirable properties not easily found in diffusion gradients [327]. One of these is defect regulation: When the source of a diffusive gradient is removed, through surgery, the pattern must decay. On the other hand, reaction-diffusion models are able to reform a new source when the old one is removed, with the maintenance of the initial polarity. Some proportion regulation is also observed: The model can accommodate a certain degree of change in the overall size of the embryo, both in terms of retaining the capacity to form a monotonic gradient, and in preserving the original proportions of the embryo to some extent. There is of course a limit: As the size increases sufficiently, a complete wave or more can fit into the domain, so that higher eigenfunctions are driven unstable. In this case we leave the régime of gradient generation and enter the realm which is better treated as prepattern formation — see section 4.3.

Analytical Studies of Gierer-Meinhardt Model As for the Brusselator model (see section 3.2.3), there have been fairly extensive analytical studies on the Gierer-Meinhardt system. With particular reference to gradient formation, a bifurcation analysis obtaining an explicit analytical form for the first, half-wave inhomogeneous solution beyond bifurcation was performed

by Granero *et al.* [135], confirming the possibility of gradient formation even from an initial uniformity. Other studies have included an analysis using the synergetic techniques developed by Haken [141], obtaining the possibility of gradient formation (and temporal oscillations) on a sphere with the aid of generalized Ginzburg-Landau equations [31]; and the demonstration of gradient formation under the assumption that the inhibitor diffuses much faster than the activator [182]. A more general and rigorous mathematical analysis was performed by Mimura and Nishiura [239], treating the existence and stability of asymmetric bifurcating solutions arising from a slight preexisting source asymmetry, and demonstrating some of the general analytical techniques introduced in appendix A.1.

Extensions With further growth of the domain, higher eigenfunctions of the Laplacian beyond the first half-wave may become unstable, leading to multiple concentration peaks and an actual chemical prepattern; this situation, already referred to, will be discussed in section 4.3.1, together with aspects of the wide range of applications in various developmental situations which have been devised for the Gierer-Meinhardt schemes (for reviews, see especially [229, 233]). In the study of self-organized gradient formation, we need also not restrict ourselves to the Gierer-Meinhardt equations (which have, however, attained historical precedence and hence attracted the most interest) as the discussion of appendix A.4 clearly indicates that all reaction-diffusion schemes will generate gradients in one dimension as the first bifurcating solution beyond homogeneity.

Applications of Self-Organizing Gradients

Reaction-diffusion-generated gradients may be postulated for any developmental situation in which gradients based on simple diffusion are applied; we shall see some experimental justification for the existence of chemical concentration gradients below. For our discussion here, we note only two prominent applications of gradients as a means of specifying positional information, whose development was specifically based on reaction-diffusion mechanisms.

Regeneration in Hydra The original application of the gradient-forming properties of the Gierer-Meinhardt model was to the study of regeneration in hydra [114]; for early introductions to this organism and its potential as a model for development, see [111, 380] (see also section 2.2.1, and discussions in [10, 141]). Hydra is a small freshwater organism, a few mm long, with a 'polar structure' along its length, consisting of a sequence of different body parts, in particular with a 'head' at one end and a 'foot' at the other. Interest in this organism arises from its peculiar ability to regenerate body parts: any small piece cut from the roughly cylindrical gastric body column can form a whole new hydra, with head and foot at the appropriate ends. Further remarkable properties of hydra are revealed by transplantation experiments: for example, if part of the head region is removed and transplanted to another part of the animal, then the likelihood of formation of a new head at the point of transplant increases with distance from the original head. Near the head, secondary head growth is inhibited, whereas nearer the foot, a new head is formed by activation of cells of the hydra by the transplant cells.

Experimental observations such as the above are strongly in favour of some graded positional information mechanism, or polarity-defining gradient, operative along the hydra body axis, and

determining the orientation by some asymmetric preexisting property in hydra, as was already proposed in one of the seminal experimental papers on grafting and regeneration experiments [380]. Such a gradient, in which each point has a fixed concentration of morphogen, is not however sufficient to account for, say, head formation at one end, at a specific concentration, because the same subarea of the animal can form either head or foot upon regeneration, depending on how the section was cut. Rather, the 'morphogenetic field' must be formed anew after the onset of regeneration, in a rapid process which must involve some sort of cell communication within the regenerating tissue [113]. This requirement for a rapid response and reformation of the gradient after surgery argues in favour of a mechanism such as reaction-diffusion rather than slower simple diffusion processes. Thus the existence of at least two relevant chemical species, an activator and an inhibitor, is suggested by the experiments.

This led to the application of the Gierer-Meinhardt activator-inhibitor model to this situation [114], with some initial success in being able to account for the experimental results. For example, following excision of a section of the body axis, the model predicts that on surgical depletion of inhibitor to sufficiently low inhibition levels, the activator is able to increase autocatalytically. Inhibitor produced concurrently diffuses away, but eventually the inhibitor builds up sufficiently to limit activation, and a stable pattern is reformed, in which the level of activator is again high enough to stimulate head formation.

Subsequent experimentation revealed (as might be expected) that further refinements of the model were needed. In particular, a modified reaction-diffusion model demonstrating proportion regulation has been devised to account for some of the observed regulating behaviour (for a description of this model and more recent aspects of hydra patterning, see [34]). Furthermore, extensive experimentation has led to the identification of at least four molecular morphogenetic substances, distributed in two pairs of opposing gradients, and involved in hydra patterning; the chemical nature of these substances is being characterized. Two of the substances, head activator and foot activator, are low molecular weight peptides, whereas head inhibitor and foot inhibitor are nonpeptide species. A more subtle reaction-diffusion model which attempts to account for the more complex and realistic interactions between these species, in particular the head activator and inhibitor, and their sources has been proposed [187], and work is continuing (see also [87]). Such developments build on the original Gierer-Meinhardt model, but do not invalidate it as an important first approximation, as the process of successive refinement of theory in the light of improved experimental tests is part of the development of any theory. It is clear that the presence of gradients of chemical concentrations of activating and inhibiting substances and their nonlinear interactions, in order to provide positional information, is well-established.

Patterning in *Xenopus* Retina A second application of reaction-diffusion theory as a means of generating gradients is to the determination of axes and polarity in the *Xenopus* retina [324]. Transplantation experiments, in which the developing retina was excised and replaced in various orientations at different stages of development, indicated that the polarity of a transplanted retinal axis, as indicated by the developing retinotectal connections, is determined by the host polarity until a critical time t_0, beyond which the internal polarity is retained irrespective of the influence of the host [234]. This situation has been modelled by assuming that the polarity is determined by the gradient of some chemical substance, for example an inhibitor; and that the surrounding tissue imposes on the retina a slight asymmetry such as the shallow gradient of an inhibitor. The external asymmetry is assumed to determine the polarity of the retina,

and to be amplified autocatalytically, until eventually the magnitude of the internally generated gradient exceeds the magnitude of any asymmetry that surrounding tissue can impose; at this point, polarity can no longer be reversed by surrounding host tissue, and the retinal polarity therefore appears determined upon transplantation.

This process was initially simulated by Meinhardt and Gierer [234], and studied further by Shoaf *et al.* [324]. In the latter investigation, the behaviour of the Gierer-Meinhardt model and that of another reaction-diffusion system, proposed by Kauffman *et al.* [180] (see section 4.3.3) were compared, in terms of the requirements that needed to be placed on the initial perturbation to be sufficient to generate the primary gradient, the stability of the gradient to changes in disc size, and the removal of various parts of the disc, modelling the loss of cells from the morphogen-producing cycle due to cell death or differentiation. The Gierer-Meinhardt equations were found [324] to be remarkably stable to a wide range of perturbations, displaying promise for realistic applications to modelling the formation of gradients, while the model of Kauffman *et al.* was considerably more sensitive to shape perturbations, as expected from the application it was initially proposed for, namely the generation of a sequence of transient patterns [180].

Self-organizing systems, such as reaction-diffusion systems, clearly demonstrate potential for generating gradients for the specification of positional information, and thus provide an alternative paradigm to simple diffusion relying on initial nonuniformities, for the establishment of a coordinate axis. A conceptual scheme for the generation of pattern from gradients is however not complete without a feasible means of interpreting graded positional information, which we consider next.

4.2.3 The Interpretation of Gradient Information

The concept of positional information, as already emphasized repeatedly, postulates a two-step process: first the coordinate system is laid down, possibly by one of the gradient-forming schemes already considered, and then the molecular 'address' of the cell is noted, and converted into some positional value, which influences its future development. Essentially, the complexity of the patterning behaviour depends on the ability of the cells to respond to, for example, different morphogen concentrations of the simple underlying chemical gradient. Thus the existence of a plausible interpretive scheme is crucial to the feasibility of such a model for positional information.

In principle, there are two ways in which the interpretation of positional information could work [381].

- The simpler method involves essentially an *isomorphism* between positional values and their interpretation: the continuous gradient in positional information is expressed directly as a continuous gradient in some cellular property. For example, the concentration of some morphogen could directly influence some cellular characteristic, such as adhesivity, the rate of some chemical reaction, or the concentration of some other substance. In this case, no additional patterning occurs beyond the simple gradient already inherent in the system.

- The second mode of interpretation is the interpretation of the continuous positional values in a *complex, discontinuous manner*. In this case the intricacies of the cell response determine the final pattern, which is qualitatively different from the initial gradient. Such

interpretation must involve **thresholds** at particular positional values; a threshold separates two qualitatively distinct regions of cellular behaviour. The effect of a threshold is an irreversible change or determination of the state of the cell, such that the new state persists even when the initial interactions which brought about the regionalization in first place are no longer present; thus thresholds and *memory* are closely connected.

The first of these modes of interpretation is essentially trivial to implement, so we concentrate on the second scheme, on thresholds.

A Kinetic Mechanism for Positional Differentiation The orthodox view of the interpretation of positional information is that, for example, morphogen-sensing genes have different concentration thresholds that may be sharpened by autocatalysis and intergenic repression. An explicit molecular mechanism for such positional differentiation in the presence of a gradient of some morphogen has been proposed by Babloyantz and Hiernaux [8, 12, 13], to complement their mechanisms for the establishment of a gradient through chemical kinetics coupled with diffusion or active transport (see also the discussions in [10, 276]). In this model, the existence of a graded distribution of a morphogen S, generated possibly by one of the diffusion or reaction-diffusion mechanisms discussed above, is assumed; S is postulated to act as a regulator at the genetic level in the synthesis of a protein E. The important requirement for the model is that the E–S relationship be nonlinear, of sigmoidal or S-shape, so that there is a threshold value of S for which considerable enhancement in the synthesis of E occurs. The model they propose for such a response curve is based on bacterial induction mechanisms of the Jacob-Monod type, involving the interactions between operators, inducers and repressors; an explicit formulation for such an irreversible scheme is given [12, 13].

Numerical solution of the model equations, assuming discrete diffusion to couple adjacent cells, indicates that a smooth variation in the gradient of the morphogen may generate a very discontinuous response curve for E, with the existence of a threshold concentration S_c for the synthesis of E, such that cells with $S > S_c$ synthesize appreciable quantities of E and are hence assumed to differentiate, while those with $S < S_c$ remain undifferentiated. This thus constitutes a kinetic scheme for the generation of sharp differences, and differentiation, in the presence of gradient-mediated positional information, with the further significant property that the number of cells in which change is induced depends critically on the length of the system and the boundary conditions.

A problem with this model, however, is that even though there is a sudden increase in the E concentration, there is no finite jump, so that cells with S just greater than S_c will have only small E. The point of differentiation is thus not well-defined in the model, on a small scale. In general, such kinetics, based for instance on the cooperative properties of allosteric enzymes, which can cause a steep shift in the level of an equilibrium between different forms of an enzyme as the concentration of some control molecule changes, are not sufficient to account for thresholds as they form essentially equilibrium phenomena, with a smooth relationship between state and position [327]. In order to obtain a strict discontinuity, feedback loops are required for the positive reinforcement that will make a certain 'choice' irreversible, and some sort of switch mechanism, such as a bistable reaction system with a finite jump at the threshold, is needed.

Bistable Kinetics in the Creation of Thresholds Such threshold behaviour may be obtained in a dynamical model, in which the cell is considered as a kinetic system, with chemicals reacting far from equilibrium; in this view a stable cell type may be interpreted as an attractor for the underlying dynamics, and differentiation related to the coexistence of and transitions between finite domains of attraction for the cell types [86]. Such a system has been suggested by Lewis *et al.* [205]: consider for example the linear activation of a gene G by a signal substance S, where G is further activated by its own product g in a sigmoidal fashion, giving a positive feedback; and g is degraded at a rate proportional to its concentration. The elimination of numerous intermediate reactions allows us to sum up the behaviour in a single equation for g:

$$\frac{dg}{dt} = k_1 S + \frac{k_2 g^2}{k_3 + g^2} - k_4 g, \tag{4.17}$$

where the k's are constants. This equation has the property that for low values of S, it has two stable steady states, whereas for sufficiently high S, above some threshold value S_c, there is only one.

Consider what happens as the signal concentration rises from $S = 0$; we may assume that the cell starts with the gene G inactive, that is, $g = 0$. Then for $S < S_c$, g remains on the lower steady state, whereas as S passes its threshold value S_c, the lower steady state disappears, and there is a discontinuous jump in g to the other steady state. As S is decreased again, the system remains on the upper steady state. Hence this bistable kinetic system displays memory, or *hysteresis* effects, which we recall is a property of self-organizing systems. (Strictly speaking, however, this mechanism for thresholds cannot be considered as self-organization, as the symmetry-breaking depends on variations in an external control parameter, S.) This switching mechanism thus provides a scheme for sharp differences and threshold responses at specific morphogen concentrations, and thus serves its purpose, which is fundamentally that of a plausibility demonstration, that such interpretive schemes for positional information *are* feasible. An example of possible bistable switching and the stabilization of developmental decisions is provided by DNA methylation.

The Applicability and Validity of Threshold Schemes The interpretation of positional information depends thus on the sharp differential response of some gene or its product to graded morphogen concentrations, with positive feedback loops to ensure the maintenance of the state. Any mechanism for regional specification in fact entails the existence of thresholds: for example, in pattern formation based on prepatterns, cells can respond by differentiating if the concentration is above or below some threshold (see section 4.3); or analogously, thresholds in for example adhesivity or mechanical stress induce certain cellular behaviour (see chapter 5). This underlines the importance of interpretive schemes for coordinate or other information that needs to be 'interpreted', and where lasting distinctions (memory) between cell types must be maintained well after the temporary signals on which they were based have disappeared. For positional information mechanisms in particular, however, there is the added situation that there is no complexity in the underlying prepattern, so that multiple thresholds must be assumed, with for example different genes responding to different concentrations of the morphogen. In principle, this could permit patterns of unlimited complexity, as every cell could respond differently to a different concentration threshold; the feasibility of such a scheme depends, however, on the precision to which both the laying down of the coordinate gradient and the interpretation of the concentration information can occur.

Lewis and coworkers [205] estimated an approximate intracellular concentration variability and used it to obtain an estimate for the expected fractional error in the specification of position, and thus for the reliability of a threshold mechanism for positional information; their estimate was was that a single gradient could specify as many as 30 distinct states reliably, although the gradient would need to be rather steep. Such precision, besides being somewhat optimistic, is probably unnecessary, however. Successive refinement of positional information through *hierarchies* of nested gradients or other mechanisms, leading to progressive determination of position or other cell properties, such as successive restriction of gene expression, is a more than sufficient, and more robust, mechanism for positional specification: a crude global 'address' is given first, and finer details are then established through local interactions — the early regional specification of *Drosophila* provides an excellent example (see section 2.2.3, and section 4.2.4 below). Such hierarchical specification could be considered as a serial combinatorial code of positional values [159, 178]. It is not necessary to construct a global map whereby every cell knows its coordinates in the entire embryo; all that is needed is knowledge of position within the local developmental field, together with a memory of the previous discontinuities and specifications that assigned the cell to that field in the first place.

4.2.4 Evidence for Gradients

The concept of gradients in the concentration of some chemical as a means for transmitting positional information has received a tremendous boost in recent years with the discovery of the graded concentrations of some chemicals that have been shown to be crucial for development (for an introductory discussion, see for example [379], and for an excellent general overview, [1]; see also [87, 150]). We have already seen above the identification of (putative) head and foot activators and inhibitors in hydra; possible candidates for mesoderm-inducing agents and other factors related to the action of the amphibian 'organizer' region have also been proposed (see for example [176]). The two most prominent candidate systems for the existence of morphogens, that is, chemicals whose concentration variations *direct* the process of pattern formation, are however found in the early *Drosophila* embryo and in the chick limb.

Gradients in *Drosophila*

The extent of knowledge of the molecular genetics of early *Drosophila* development probably renders this the best-understood developmental system. The homeotic genes responsible for laying down the ground plan of the fruit fly have been identified, and their gene products characterized and their ranges and periods of expression established. As a consequence, much is known about the details of the establishment of patterns both along the anterior-posterior and the dorso-ventral axes (for detailed introductions, see for example [77, 200]; an excellent recent review is given in [314]).

A brief outline of the most significant features of *Drosophila* patterning is given in section 2.2.3. From this discussion, the extensive feedback controls and mutual regulatory interactions between genes and their products to control each other's expression is apparent. Most pertinent, however, is that *Drosophila* provides a clear paradigmatic example of the successive specification of positional information by a hierarchy of positional signals, through the five-tiered system of genes — egg-polarity, gap, pair-rule, segment-polarity and homeotic selector genes — which

successively subdivide the early embryo. In this way, first global coordinates, and then ever finer local 'addresses' are laid down [1].

The *bicoid* Gradient Of particular interest in the context of gradients is the experimental observation of the exponentially graded expression of gene products, especially of the protein encoded by the *bicoid* gene, as already indicated in section 2.2.3. Strands of maternally-produced RNA are harboured in the cytoplasm near the prospective head end of the egg before it is laid, thus predetermining the polarity of the embryo. Once the egg is laid, it starts to synthesize the *bicoid* protein, which diffuses along the egg, setting up a concentration gradient running from head to tail. By staining the embryo with an antibody against the *bicoid* protein, this gradient can be observed. This gradient controls the position of the boundary between the fly's head and thorax and also activates other genes at specific positions along the embryo, thus turning on further chemical gradients in hierarchical sequence to specify successively detailed positional information and eventually activating the homeotic selector genes which trigger the genetic pathways controlling the identities of each of the body segments of *Drosophila*.

Many of the predictions of a simple diffusion-based mechanism are borne out for *Drosophila*. For example, eggs with multiple copies of the *bicoid* gene display higher concentrations of the protein throughout its graded expression, and a resultant distortion in the segmentation pattern, with the segments being displaced towards the tail end of the embryo; this is as expected from a threshold mechanism where gene expression occurs at a specific threshold concentration of the morphogen. Other predictions based on a morphogen gradient mechanism are also fulfilled, such as a segmentation distortion in the absence of *bicoid* protein, and a reversal of polarity if cytoplasm from the head part of a normal egg is injected to the tail of an egg that has no *bicoid* of its own — a head develops at the site of the injection. Early *Drosophila* development is somewhat unusual in the extent to which its polarity and pattern formation are controlled by maternal cues, but the results on *bicoid* indicate incontrovertibly that cells *can* respond to graded differences in chemical concentrations, and hence demonstrate the important role of morphogen gradients in providing positional information and playing a fundamental role in developmental pattern formation.

Anterior-Posterior Patterning in the Chick Limb

The classic example of a gradient situation in vertebrate development is that of pattern formation in the chick limb, which we have already encountered in section 2.2.2, and in particular the controversial role of retinoic acid as a possible morphogen. Pattern formation along the anterior-posterior axis appears to be controlled by a positional signal, which originates at the posterior end of the wing (or leg) bud, in a group of cells known as the *zone of polarizing activity* (ZPA). This region has the peculiar property that if it is transplanted to the opposite, anterior, end of the limb, duplication of structures occurs. The resultant structures depend on the position of the graft with respect to the original polarizing region, and the number of cells grafted, to the extent that a graft of an entire ZPA to the opposite end of the bud can cause mirror-duplication of bone (especially digit) structures. The properties of the ZPA stimulated the theoretical proposal that its action was due to the diffusion of a morphogenetic substance emanating from the ZPA, whose concentration was highest at the posterior margin, and decayed towards the anterior end to produce an exponential concentration gradient (see [376]).

Retinoic Acid — a Morphogen? This theory received a major boost when it was found that small beads, impregnated with *retinoic acid*, could mimic the effect of the ZPA, in its potential for the specification and duplication of structures; retinoic acid diffuses out of the beads and sets up a concentration gradient across the limb [350]. The case for retinoic acid as the natural morphogen was further enhanced by the discovery that the limb bud contains endogenous retinoic acid, in a graded distribution from posterior to anterior, at concentrations corresponding to the known induction thresholds for the specification of the different embryonic digits [326, 343]; and that there is furthermore a graded distribution, with opposite polarity, of a cellular retinoic acid-binding protein (CRABP) which is assumed to act as a receptor for retinoic acid and reduce its free concentration reaching the nucleus to a level appropriate for the differential regulation of gene transcription [212]. These factors all encouraged the logical interpretation, that the implant of a retinoic acid bead was simply mimicking the natural effect of the ZPA in creating a gradient of retinoic acid, and that retinoic acid was thus the natural form-producing chemical species, or morphogen, in the limb bud.

Another interpretation has however been mooted recently [43]; that retinoic acid from the bead could cause cells near the implant to change their character and become a new polarizing region, which could then direct pattern formation by some other mechanism; in this view, retinoic acid would play a fundamental role in its ability to trigger the creation of a ZPA, but would not necessarily be the actual morphogen that directed pattern formation. Two different teams have come to this conclusion by different routes: by considering the ability of retinoic acid to induce a neighbouring polarizing region [361]; and by monitoring the activity of a gene sensitive to retinoic acid, in the vicinity both of an implanted ZPA and an implanted retinoic acid bead, with the conclusion that the natural mode of action of the polarizing region is not to release retinoic acid [281]. The complexity of the biochemistry of the limb bud means that the experiments are, however, open to different interpretations, so that retinoic acid, or some close chemical relative, may still be the best candidate for a vertebrate morphogen.

It is of additional interest to note that the chick limb holds further wonders: for example, there is a family of homeotic genes, denoted *Hox-4.4* to *Hox-4.8*, which are expressed in graded sequence across the limb, corresponding to the positions of the different digits, with different positions across the bud expressing different combinations of the genes. The intriguing aspect is that the spatial expression patterns of the genes correspond to the spatially ordered sequence of the genes along the DNA, so that in this case, there is an isomorphism between DNA structure and expression, the basis and control of which is a further mystery (see for example [349]).

What the chick limb studies indicate to us is that even the most seemingly clear-cut gradient situation, where an endogenous chemical gradient has been identified and agrees with modelling predictions (such as the LSDS diffusion model), and which can be mimicked experimentally, with analogous results to the unperturbed case, is still not a definitely established morphogen situation. The theory in this case has been highly successful, and has served to stimulate further investigations of gradient and other positional information mechanisms, as well as the search for other biochemical gradients; but the intricacies of developmental situations always far surpass anything that might be encompassed, especially predictively, in theory — there is no substitute for experimentation and the rigorous testing of predictions.

4.2.5 Non-Gradient Mechanisms for Positional Information

We have investigated the formation of and evidence for graded concentrations of chemicals, whether established purely by diffusion or by a combination of reactions and diffusion, in some depth, in our consideration of the specification of positional information along a single Cartesian coordinate axis. While the above models are the most-studied and best-supported, there are in principle other means of establishing a linear coordinate system not involving diffusion-based graded chemical concentrations. We shall only mention some of these schemes briefly:

Other Transport and Signalling Schemes

Active Transport First of all, there is no explicit need for morphogen transport to be due to passive diffusion; clearly, active or facilitated transport of appropriate chemicals could also give rise to polarized phenomena and concentration gradients. Such models assume an asymmetry in the cellular properties, that is, a preexisting polarity, which is manifested more clearly in the resultant concentration gradient, opposite to the direction of the morphogen's transport (see [276]). For such a scheme to work, the cells on at least one boundary need to play a privileged role. A model involving non-polarized cells but based on a molecular mechanism whereby the gradient of the morphogen is established by active transport between a source and a sink has been elaborated by Babloyantz and Hiernaux [12], together with a discussion of the interpretation of this gradient information; this work formed a complement to the self-organizing diffusion-based models described above [13].

Contact Interactions Gradient formation does not explicitly require the propagation of a substance along a field: Babloyantz has considered [9] the influence of one cell on its neighbour through surface contact interactions in which molecules in the surface of one cell influence the rates of reactions in neighbouring cells, through for example activation or inhibition mechanisms involving membrane-bound enzymes and metabolites, without the actual passage of molecules from one cell to the other. Such systems exhibit all the self-organizing properties of reaction-diffusion systems [9]. The ability to generate positional information through contact-mediated communication is significant, in the light of the importance of cell contacts, in adhesion, contact inhibition and other membrane-facilitated interactions (some of which we shall encounter in the next chapter), and provides a further possibility for symmetry-breaking behaviour.

An earlier model for intercellular communications at the membrane level through cell contact [227], which was applied particularly to the mechanism used by cells in the pseudoplasmodium of the slime mould, *Dictyostelium discoideum*, to determine their position, assumed a preexisting cellular polarity in the form of different contact-sensing molecules on the front and the rear of cells in the developing field, and was thus not self-organizing.

Waves and Timing Signals

A Phase-Shift Model The first proposed model for the establishment of a graded property that might transmit positional information was the phase-shift model of Goodwin and Cohen [128]. In this model, it was assumed that cells function as autonomous oscillators, under the influence of a pacemaker cell. This specialized cell (again an initially polarized field is assumed)

is considered to emit two signals of equal frequency, the second propagating more slowly than the first. As the signals move through the field, the phase-angle difference between them increases linearly, giving rise to a gradient in phase differences which is read by the cells and thus allows the conversion of the temporal organization of the individual cell into a spatial ordering, that is, positional information. The proposed mechanism requires a sequence of complex hypothetical biochemical reactions, however, and has little experimental support [377].

Waves in Positional Information Other, more attractive mechanisms for specifying positional information are based on intercellular signals or time-based mechanisms. As postulated in the phase-shift model above, signals can be transmitted along a line of cells, through a wave in some chemical concentration, functional coupling through entrainment of oscillations, or by some other mechanism. If, then, the cells in an array somehow 'record' the time of arrival of a wavefront passing by, the sequential passage of the wave will be converted into a gradient in arrival times, providing a source of positional information. Internal timing or counting mechanisms may readily be envisaged, for example by measuring the concentration of some chemical whose production ceases on arrival of the wave, or a count of the number of completed cycles of some intracellular oscillation. It is clear, however, that any such wavefront mechanisms require the existence of some specialized region acting as the source of the wave, and thus cannot embody strict symmetry-breaking properties.

Reaction-diffusion mechanisms can, as we have seen, produce wave-like pulses of chemical concentrations; consider for example the threshold waves demonstrated for a substrate-inhibition reaction by Britton and Murray [42] (see section 3.2.3). The paradigmatic chemical oscillator is the Belousov-Zhabotinskii reaction, discussed in some detail in appendix B, whose visually striking behaviour, accounted for by mathematical analysis of the reaction-diffusion formulation of the kinetics, includes concentric and spiral wave patterns. Hence a mechanism based on reactions and diffusion may be responsible for the wavelike intercellular signalling which, coupled with a timer, can provide positional information. Note especially that the speed of such reaction-diffusion information transfer is orders of magnitude faster than velocities of signal propagation through pure diffusion, making reaction-diffusion mechanisms particularly efficient for such wavefront mechanisms (see appendix B.3.2).

Progress Zone Model An alternative to the physical propagation of a wave or some other signal is provided by the progress zone model [339], which again emerged from experiments on the chick wing bud (see section 2.2.2). Skeletal development along the proximo-distal axis depends on a group of cells at the tip of the bud, the apical ectodermal ridge or 'progress zone', where most of the growth takes place. Numerous grafting experiments led to the idea that the proximo-distal coordinate of the cell reflects the time it spends in the progress zone; cells presumably somehow 'count' the number of cell divisions they experience in the zone, and 'stop their clocks' once they exit the zone. Hence again, *spatial* positional information is acquired via *temporal* information, in contrast to conventional gradient mechanisms where no explicit growth or time dependence is taken into account.

An extension of the progress zone model [381] considers coupling of pattern formation along the anterior-posterior and proximo-distal axes of the chick limb, in which autonomously oscillating chemical reactions occurring within cells in the progress zone give rise to a prepattern

in concentration peaks in the anterior-posterior direction (established for example through a reaction-diffusion mechanism — see section 4.3.4), with the number of peaks determined somehow by the time spent in the zone. Here we thus have a hybrid of a positional information mechanism and a prepattern mechanism [159]; the coordinate information instructs the type of pattern to be generated through the prepattern mechanism.

Clock-and-Wavefront Model A last model that should be mentioned here briefly, mainly in the light of its historical origins in catastrophe theory (see section 6.1), is the clock-and-wavefront model of Cooke and Zeeman [64]. Here no new mechanism for the establishment of a gradient is proposed, but rather, some morphogen or other gradient, produced by unspecified means, is assumed to span an array of cells. The model was established to account in particular for the sequential formation of vertebrate somites. Here the gradient is assumed to specify the rate at which cells progress towards their act of segmentation; it may be considered to introduce an 'alarm clock' [159], set for a time corresponding possibly to the morphogen concentration. Furthermore, each cell is assumed to contain a synchronously oscillating biochemical oscillator, the 'clock'. The idea of this scheme is that the alarm clocks could later ring in sequence from one end of the array to the other, causing the oscillator clocks to stop and resulting in a periodic pattern through phase coupling between the two oscillations. The pattern is manifested, for example, in periodic variations in adhesiveness due to the superposition of some gradient-based and oscillating concentrations, which leads to aggregation of cells, producing segmentation.

This scheme was devised to account for the experimental failure to interrupt the passage of somitogenesis by surgical barriers or cuts that would block physical signals; the wavefront (or 'secondary wave') refers to the visible process of segmentation that sweeps from one end of the field to the other, without any intercellular communication at that stage. This model is presented here briefly as a further example of the possible application of positional information or gradient mechanisms in combination with other mechanisms to produce pattern; it will be referred to again in chapter 6, where its conceptual origins in catastrophe theory will be assessed. Its major drawback is that, beyond a conceptual level, it has never really been spelled out in detail or properly tested [327].

We have here considered a range of models, all connected with some form of gradient formation or positional information, but in general requiring the assumption of preexisting asymmetries or polarities, and thus not falling into the category of self-organizing mechanisms. Nevertheless, this discussion illustrates the wide variety of proposed mechanisms and applications for positional information mechanisms along a single array of cells that exists. It thus serves, in conjunction of course with the simple diffusion and reaction-diffusion-based gradient mechanisms and the strong experimental support for gradients, to highlight the widespread acceptance, generality and explanatory power of the positional information scheme first proposed by Wolpert, which has been dubbed the "currently reigning theory" [159, p.7] of pattern-formation models.

4.2.6 Polar Coordinate Model

As we have considered in some depth, the conventional description of embryos and limbs is in terms of anterior-posterior and dorso-ventral axes, generally implying a Cartesian system of

orthogonal coordinates for the specification of positional information. From a purely mathematical perspective, however, transformations to other coordinate systems are clearly possible. In the developmental context, the coordinate system chosen is however not arbitrary, as different systems would embody different mechanisms of specification; and we have already encountered (see section 4.2.4) some of the ample experimental evidence that, in some developmental contexts at least, gradients of the concentration of some molecule *do* in fact exist and thus provide more than just a useful heuristic aid for understanding how one-dimensional positional information may be specified. The laying down of gradients may also proceed by a self-organizing mechanism such as reactions coupled with diffusion (see section 4.2.2), thus constituting a link with our overall concept of symmetry-breaking. Gradients may in some sense be thought of as a *non-local* mechanism, as although concentrations are read off locally by the cells, the generation of a gradient does require a long-range diffusible signal.

A Polar Coordinate Account of Regulation

Regeneration Experiments In contrast to such a rectangular coordinate system stands the **polar coordinate model** (PCM), mathematically a clear alternative for specifying the position of a point in a domain. This model was developed initially [105] to account for the results of numerous regeneration phenomena observed after experimental manipulations such as excisions and transplantations in the limbs of amphibians and cockroaches, as well as in imaginal discs in *Drosophila*. Following such an intervention, *regulation* may result in regeneration of the missing parts as well as the generation of wholly new structures, depending on the conditions (see section 2.2.2 for an introduction to regeneration phenomena). Regulation can in general occur in two ways:

- By *epimorphosis*, in which new pattern elements are added through growth, with little change in the existing parts (this is most common in secondary embryonic fields, that is developmentally autonomous regions of the embryo such as limb buds and imaginal discs); or

- By *morphallaxis*, in which the remaining parts of the *field* (the region in which regulation can occur in response to surgical manipulation) are remodelled, with little growth, to form a miniature but complete pattern.

A wide variety of grafting experiments has been carried out, and some remarkable abnormalities have been formed (some of which are in fact also observed, rarely, in nature). A typical example is the amputation of a presumptive right limb bud or blastema (of, say, a newt or salamander), which is grafted onto a left limb bud. The most common result of such a *contralateral* graft is the development of the blastema into a right limb, but accompanied by two supernumerary (additional) left-handed limbs. Numerous variations on the general theme have been performed, such as rotation or even inversion of a limb before grafting, and the resulting regeneration or intercalation phenomena have been observed. The polar coordinate model proposed by French, Bryant and Bryant in 1976 [105], a conceptual model designed to provide a basis for understanding predominantly epimorphic regulation, was a triumph in terms of unifying and accounting for a wide variety of previously unexplained observations.

Formulation of the Polar Coordinate Model Briefly, in the polar coordinate model, also referred to as the clockface model, position in the developing limb or imaginal disc is specified by a radial coordinate corresponding to the proximo-distal axis, and an angular coordinate, measuring angular position in a plane perpendicular to the proximo-distal axis. For convenience, these positional, or field, values have been discretized, and the angular component was (arbitrarily) assigned values running from 1 to 12 = 0, as on a clockface; no discontinuity was implied at the 12/0 point. The positional values need not be evenly spaced; the spacing of circumferential values is deduced from the intercalation behaviour displayed in various grafting or fragmentation experiments. Only two dimensions of positional information are specified in this model, which is clearly justified in the case of imaginal disks and the epithelial sheets in which insect appendage patterning occurs; in fact it was hypothesized [105] that patterning in general occurs in two dimensions rather than three, and that the three-dimensional morphology of embryos occurs by folding, shaping and growth of cell layers, coordinated for instance by mesenchymal-epithelial inductive interactions (see chapter 5).

The polar coordinate model is developed in terms of a set of purely formal rules chosen to account for the observations; no direct molecular interpretation or mechanism is implied by these rules, which make no reference to cellular composition or inheritance, but are of a purely relational nature. The first rule is the **shortest intercalation** rule: when normally nonadjacent positional values are confronted as a result of grafting or wound healing, local growth occurs at the junction until cells with all the intermediate positional values have been intercalated; then growth ceases. For the case of circumferential values, the intercalation occurs by the shortest route, so that if say the values 1 and 4 are juxtaposed, the intercalation will recreate the sequence 1, 2, 3, 4 rather than take the longer alternative route, 1, 12, 11, 10, 9, 8, 7, 6, 5, 4. Thus if an imaginal disc, for example, is fragmented into two unequal-sized parts, with positional values of say 3 and 7 at the ends, the smaller (already containing 3, 4, 5, 6, 7) will *duplicate*, while the larger will *regenerate* fully and form the complete imaginal disc structures (as is in fact observed).

The second rule was originally proposed as the **complete circle** rule for distal transformation [105]: if a complete circle of circumferential positional values exists at some proximo-distal level, cells with all the more distal positional values are produced. Subsequent experimental evidence for distal transformation occurring when not all the circumferential positional values were present, together with theoretical reservations about the non-local character of this rule (the behaviour at a point must depend on long-range interactions to verify the completeness of the circle) led to a reformulation of the polar coordinate model in terms of purely local interactions [46], by replacing the complete circle rule by the **distalization** rule: if intercalation and growth leads to cells with positional values identical to those of adjacent pre-existing cells, the new cells will adopt a more distal value. This *local* rule leads to the same predictions as the previous complete circle rule; in particular, a complete circumference of positional values will lead to a distally complete regenerate.

Applications of the Polar Coordinate Model These two rules together provide a purely phenomenological description of regulation, which has been able to account for a wide variety of experiments through this simple and unified interpretation, although there remain some problems, and exceptional experimental observations not fully covered by the model (for discussions, see for example [46, 103, 104, 105, 159]). The impact of the model has also extended well beyond

the initial restriction to epimorphic regulation, and has been brought to bear on more general developmental and evolutionary studies: Investigations of the interactions between developing limb buds and regenerating limb blastemas in amphibians suggest that similar patterning mechanisms are at work in these two systems [243]; thus the polar coordinate model may well be applicable to the original generation of structure in limb buds, imaginal discs and other systems [365]. Insofar as the polar coordinate model is applicable to normal development, the morphologies it is predicted to produce can also produce *constraints* on the possible forms that could have evolved for animals using this mechanism. The correspondence between the predicted and observed patterns has been analyzed for the particular case of vertebrate digit patterns, and a high degree of consonance between theoretical predictions and the skeletal makeup of the hands and feet of tetrapod vertebrates was observed [163].

The Polar Coordinate Model and Generalized Fields Numerous authors have pointed out (see [103]) that the essence of the model is its preoccupation with purely local interactions in such a way as to remove discontinuities in positional values. Apart from the fact that the choice of twelve discrete circumferential values is somewhat arbitrary, no particular coordinates are required at all, if the map in, say, the insect epidermis tends to reestablish *continuity* after a disturbance. Then the mechanism underlying the polar coordinate model would be a general *smoothing* mechanism, which would recreate structures after disturbance independently of the coordinate system. Such spatial smoothing properties of this phenomenological model have led some investigators to consider generalized *field* approaches with suitable continuity properties, by analogy with physical fields, in order to provide a mathematical foundation for the model (see for example [126, 352]; also refer to chapter 5.3.4). It appears, however, that nonspecific models based only on continuity properties do not yield definite predictions for many grafting experiments, so that more precise models, based on a particular coordinate system, may in fact be needed (see [103]), and the polar coordinate model has in general fared well, with extensive supporting evidence from insect, amphibian and also chick limb experiments — this also implies that vertebrates and arthropods may use a common patterning strategy [159].

Molecular Basis of the Polar Coordinate Model The nature of the interactions underlying the polar coordinate system and regulation of the positional values was left unspecified in the original formulations of the model [46, 105], which purely specified phenomenological rules. It has of course been a concern to attempt to justify the model by placing it on some sound molecular footing.

In such a quest, of interest firstly is that entirely local interactions are required [46, 365], dispensing with the long-distance diffusion gradients involved in Cartesian positional information (although we did note that cell-contact interactions could generate similar gradients [9]). Local interactions hold more promise for stability and robustness, in view of the immediate feedback possible in the face of perturbations. They suggest that 'positional value' is a property of the cell surface and its interactions with its neighbours, rather than of the intracellular concentration of some diffusible morphogen. Thus we would expect cell-cell adhesions and interactions, possibly mediated by cell adhesion molecules (CAMs) (see section 2.1.2) or local transport through gap junctions, to be crucial to patterning, and defects in, say, adhesivity to lead to defective growth, an effect that is in fact observed. To pinpoint local contact-mediated interactions as being the basis for the positional information is not nearly the same as indicating how the pattern or

coordinate system is set up, or how the different circumferential positions are specified, however, so there is still a mystery to be solved.

Circumferential Coordinates from Orthogonal Gradients It has been pointed out that coordinate systems resembling polar coordinates may be established by measuring the ratio between the concentrations of two morphogens (see [159]) — although it is not clear how this might be achieved — or more directly, by the use of two orthogonal (more generally, transverse) gradients or Cartesian coordinate systems. Provided there are at least three thresholds at which these gradients are interpreted (see section 4.2.3), so that every coordinate direction is subdivided into four compartments, the peripheral compartments may be labelled as for a (square) clockface, thus enabling polar information to be specified by means of gradients, for which experimental support already exists [300].

A similar interpretation has been proposed by Meinhardt, who deals with the tricky question of how the presumptive polar and Cartesian coordinate systems mesh, for example in *Drosophila* where, as we have seen, there is a profusion of experimental and theoretical evidence for gradients and linear patterning along the anterior-posterior and proximo-distal axes; how does this correlate with polar coordinates in the imaginal discs? He proposes that the primary patterning creates *compartments* of differently determined cell types with *boundaries* which interact, for example through a morphogen whose production depends on both compartments. Two intersecting borders due to patterning and compartmentalization in transverse directions, which require a common point to exist for three or four distinct sectors, allow the determination of a 'handedness', since this is independent of whether 3, 4 or 12 angular positional values are used.

The 'cooperation' of these compartments as organizing regions for secondary embryonic fields then provides a molecular basis for the polar coordinate model and enables analogous predictions to be made, and some violations of the polar coordinate model may also be successfully accounted for [230, 231]. This scenario accounts for the relation between the rectangular and polar coordinate systems, as well as for the original creation of the complete circle of angular coordinates which was merely postulated as an initial condition in the polar coordinate model, but here appears as a straightforward consequence of the interaction of compartments produced by the primary patterning.

The model of Meinhardt thus deals with hierarchical patterning, with borders separating regions of different determination arising from interpretation of the primary positional information; at these borders, in turn, positional information is generated for the next finer developmental subdivisions, and so on [230, 231] (see also [233]). Note that any mechanisms, such as the two last-mentioned, which account for polar coordinates in terms of transverse gradients, are able to utilize any of the gradient-forming mechanisms discussed above, including self-organizing reaction-diffusion mechanisms such as the one proposed by Gierer and Meinhardt [114], so that a potential link with self-organization exists.

Molecular Evidence for Polar Coordinates In the absence of any experimental information specifically in favour of polar coordinates, the above models accounting for the formation of a polar coordinate system in terms of orthogonal Cartesian axes or interaction between boundaries, models which have an accepted molecular basis, appear attractive. In this case, however, polar coordinates have the status of being merely an epiphenomenon, the consequence of a par-

ticular configuration of linear gradients, rather than constituting an independent developmental mechanism. Furthermore, the molecules involved in Meinhardt's scheme are unfortunately not identified, and no means is given of identifying them. Without a molecular foundation, the polar coordinate model has remained until recently a purely formal structure [365].

Recently, however, there has emerged evidence that the expression patterns of certain genes in the leg imaginal disc are confined to certain radial coordinates, while others are unequally distributed in an angular direction [45]. The *wingless* segment polarity gene, for example, is expressed in a narrow ventral-angular sector in the disc [65]. Many of the genes involved have also been shown to be crucial for leg patterning. The segment polarity genes act in the primary patterning of the *Drosophila* embryo to specify position along the anterior-posterior axis (see section 2.2.3); in a similar manner, localized expression of *wingless* and other segment polarity genes, it is conjectured, could specify radial and circumferential coordinates in the leg disc. Furthermore, these genes probably participate in specific signalling pathways in the early embryo; as the positional information system in the imaginal discs appears to utilize many of the same gene products as the initial axis specification, many of the same pathways of communication could also be used [45]. Work such as this, which is very recent, could enable progress to be made towards understanding the genetic basis for the cell interactions which underly the polar coordinate system of positional information. In particular, such evidence points towards the polar coordinate system as being an *independent* concept, not merely a manifestation of certain interactions of Cartesian gradients.

The polar coordinate model has excited a great deal of interest as a paradigm for pattern formation, especially in view of its excellent predictive correspondence with experiments, which we have hardly touched on. It is furthermore in no way in conflict with longitudinal coordinate systems and gradient models, as these might operate independently in different embryonic or regulatory contexts. We have paid some attention to this highly interesting and important model for positional information, even though it does not strictly qualify as self-organization (beyond the possible links with orthogonal reaction-diffusion-specified Cartesian coordinates, and the connection with generalized field models — section 5.3.4); the circumferential polar coordinate system of positional information is presumed to be set up as an initial condition to the model, and its origin is not queried.

We have thus considered the two main classes of positional information models, Cartesian and polar models; for both, the problem is in how 'simple' information can generate a complex response. We treat next the essentially converse problem, the establishment of a complex prepattern requiring only a simple binary on/off response.

4.3 Chemical Prepatterns

Pattern formation as a two-step process — with cells responding in a slaved fashion to chemical inhomogeneities, rather than participating intimately in the patterning mechanism — can take two fundamentally different forms, as we saw in section 4.1:

> The concept of positional information suggests that cells have an intrinsic record of their position that effectively gives them an address. This record, the positional

value, is used by the cells to determine cellular properties according to the cells' genetic constitution and developmental history, that is, to interpret positional value. It is as if the cells were in a coordinate system and the overt pattern emerges from the process of interpretation. Thus the pattern need not be isomorphic with the set of positional values which could be a set of graded concentrations. In principle, a specific cell type could arise sharply at any point. In contrast, a prepattern mechanism — such as for a spacing pattern — develops a distribution of morphogen that is isomorphic with the overt pattern of formed structures. For example, if a particular cell type forms only above a threshold concentration, its occurrence will correspond to the crest of the waves. [381, p.8]

Motivation for the Study of Prepatterns There are, in particular, frequent cases in embryology involving the formation of repeated structures, from the striped segmentation pattern in *Drosophila*, and somitogenesis — as highlighted in Francis Crick's much quoted comment "There is something in embryology that likes stripes" [69] — to the formation of multiple hairs, bristles and other skin patterns in a multitude of organisms. From the point of view of economy of information, it seems wasteful to have to specify unique positional information and independent thresholds for each of these structures; thus it appears unlikely that a pure gradient mechanism is involved.

The repetition of similar structures implies that at least part of their epigenetic 'coding' [159], that is the combination of mechanisms involved in their formation, is the same. Remaining for now within the paradigm of chemical concentration patterns, we thus require a mechanism that generates a spatial series of identical determined states through multiple concentration peaks and troughs, that is, inhomogeneities beyond a mere gradient — in short, a **prepattern**. Prepattern mechanisms are ideally suited for the specification of similar elements, as instead of requiring a complex interpretive mechanism in response to positional information, a prepattern mechanism allows cells to be 'stupid'; it provides them with identical signals for identical structures, and requires only that they respond to that signal, by switching to an appropriate state, usually by an on-off choice.

It is useful to clarify at once what has frequently been a source of misconception or confusion. The prepattern school of thought has been criticized for implying an infinite regression of patterns, whereby each pattern is induced by another. This criticism was historically justified while levelled against the notion of preformationism, which argued that the eggs (or sperm) contained preformed 'homunculi', or miniature versions of the adult organism, which in turn possessed eggs with further homunculi *ad infinitum*. It would today be legitimate if prepatterns could only be obtained by induction from other patterns. But our extensive studies above (see especially chapter 3) have indicated that pattern formation *de novo*, or self-organization, *is* possible. As expected, virtually all models to account for chemical prepattern formation have exploited the properties of *reaction-diffusion equations* to produce inhomogeneous spatial patterns.

Turing — Morphogens and Reaction-Diffusion Equations

We have already noted the seminal contribution of Alan Turing [354], who pointed out that the presence of diffusion in a system of reacting chemicals may induce an instability: Uniform solu-

tions become unstable to small perturbations, and stable nonuniform spatial patterns can result — see section 3.2.2; we focus here especially on applications in developmental biology. The motivation for his study was, indeed, the spontaneous creation of inhomogeneities in developmental pattern formation and morphogenesis, as evidenced by the title of his paper, *The Chemical Basis of Morphogenesis* [354]. Turing asked the question: "if a group of diffusion-coupled cells have identical molecular apparatus, are there chemical kinetics which will encourage the formation of non-uniform molecular concentrations over space in an initially uniform region? " [19, p.502].

In demonstrating an affirmative answer to this question, Turing produced a detailed model, with appropriate kinetics, to show the mode of action of his scheme. To describe the chemical species specifically involved in a reaction-diffusion or other prepattern formation system, he coined the term **morphogen**; he postulated such molecules to be involved in the generation of biological form, with subsequent cellular differentiation or other morphogenetic responses occurring in response to the morphogen pattern. Turing proposed specific biological applications for his theory, related to his analysis of the reaction-diffusion system on a ring of cells; in particular, he suggested that the stationary wave pattern on the ring could be relevant to the generation of tentacles from the head of a regenerating hydra, which has circular symmetry, and also to the development of whorls of leaves on the Woodruff plant. Towards the end of his paper, he also briefly considered the breakdown of homogeneity on a sphere, with application to gastrulation.

Responses to Turing's Theory It took some time for Turing's theory to generate the interest its originality and significance deserved [150, 202]. The initial scattered reactions from biologists were largely negative, and were directed at the prediction of a chemical wavelength depending on the kinetic constants arising from the reaction-diffusion system (see for example equation (3.34)). Such a definite wavelength was interpreted as contrary to the regulation and flexibility in pattern scales commonly displayed by developing systems in the face of size variation; Turing's chemical wavelength was taken to imply that a pattern would form only under rigid conditions, if the length of the system was exactly commensurate with the chemical wavelength. Thus Waddington dismissed the model by labelling it "inherently chancy", and likely to "play a part only in the quasi-periodic dapplings and mottings which often fill up relatively unimportant spaces" [358].

Further criticisms may be traced to the specific kinetics that Turing used, in particular their linear character, which implied a long-term instability, and an inability to guarantee non-negative concentration solutions. Thus an early numerical study by Bard and Lauder [19], while confirming the possibility of the formation of spontaneous patterns, questioned the Turing model on account of the sensitivity of patterns to variations in initial conditions, and concluded that the kinetics were too unreliable to generate regulative systems, or any situation where the number of pattern units was crucial. They did however concede the possibility of applications to mosaic systems, or to any situation where the detail of the patterns was imprecisely defined, such as zebra stripes, sea shell patterns or the spacing of skin hairs (and in the process pre-empted several later applications of reaction-diffusion systems — see below).

Lacalli and Harrison — Applications of Turing Kinetics Later studies specifically on Turing's two-morphogen linear model by Lacalli and Harrison [196, 197] focussed more closely

its regulatory capacity: The size ranges over which the simple binary pattern had the highest growth rate and was thus stable, were found to extend over a factor of at least five in the length of the system; this was proposed to have possible applications to the regulation of the ratio of pre-spore to pre-stalk differentiation in the slime mould, *Dictyostelium discoideum* [196]. Subsequent work [197] showed that in general the pattern-generating behaviour of Turing's model could be expressed in terms of two reduced rate constants k_1' and k_4', which represent autocatalytic and self-inhibitory rates, and in general include combinations of the kinetic parameters of the system as well as the concentrations of precursors of the reaction system under study. By reducing morphogenetic models such as the Gierer-Meinhardt model and the Brusselator to their linear limits, Turing's conditions [354] were then used to identify the regions in which different morphogenetic behaviours occurred, and helped to clarify the differences in the non-linear behaviour of these models (see section 3.2.2). A further application considered structure formation in the equilibrium between two enantiomers, where the optical asymmetry was taken as the appropriate morphogen concentration [153].

Such attempts at directly applying Turing's detailed kinetics have faded, however, giving way to the use of more realistic available model equations; and it has become clear that Turing's real, and revolutionary, contribution was the conceptual breakthrough of the possibility of symmetry-breaking leading to chemical and biological self-organization.

Thermodynamic Feasibility — the Brussels School General acceptance of the possibility of self-organization depended, as we have already seen, on the thermodynamic feasibility studies of the Brussels school (see section 3.1.3). The analysis of nonlinear dissipative systems far from equilibrium demonstrated that the homogeneous state could become unstable, and spatially inhomogeneous structures form; and in particular, that the pertinent conditions were satisfied by Turing's model [311]. A significant further contribution of Prigogine and coworkers was the introduction of the Brusselator kinetic model [310], discussed above in section 3.2.3, which has provided a simple molecular realization of reaction-diffusion kinetics, and the study of which has helped to elucidate many features of the solution behaviour of such systems.

4.3.1 The Work of Gierer and Meinhardt

We have already had occasion to refer to the fundamental contributions of Alfred Gierer and Hans Meinhardt in the 1970s — see section 4.2.2, and reviews in [112, 229, 233]. The interest of biologists in reaction-diffusion systems and spontaneous pattern formation was largely aroused as a result of this work [87], which revolved mainly around heuristic arguments, combined with mathematical and numerical analyses, for the development of models for pattern formation; the proposal of a range of applications in various developmental fields for their models; and in particular also their clarification of the key requirement of the combination of local activation and long-range inhibition for the generation of diffusive pattern formation [114]. The apparent similarity between their computed results and many patterns found in nature stimulated interest in the wide applicability of self-organizing mechanisms involving lateral inhibition to the study of developmental pattern formation.

Gierer and Meinhardt proposed a variety of models for activator-inhibitor and depletion mechanisms, but their names are most closely associated with a specific activator-inhibitor

model, equations (4.7)–(4.8), with or without the production term of activator due to sources. This model was initially proposed in conjunction with an application to the generation of gradients of positional information in hydra, in the case where only a half-wave pattern at the chemical wavelength fitted into the size of the domain [114]; we have considered this application in section 4.2.2. (Note: in a *depletion mechanism*, the activator is accompanied by a substance necessary for activator production, but which is depleted by the activator. The fall-off in the concentration of this substance leads to a drop in the rate of activator production, and hence stabilizes the system. This mechanism may also be modelled by a reaction-diffusion system, with similar self-organizing properties to the activator-inhibitor system [150])

Patterns of Multiple Peaks

Our understanding of reaction-diffusion equations indicates that, as the size of the domain increases, more wavelengths can be accommodated in the domain, with the generation of patterns of multiple peaks and troughs. This prediction, originally due to Turing [354], was confirmed by Meinhardt and Gierer [234] through numerical simulations, in one and two dimensions. In terms of the activator-inhibitor concept, such multiple peaks result if the range of inhibition is small relative to the total size of the system. This permits the formation of secondary peaks of activation at points where the inhibitor concentration is sufficiently low, so that the autocatalytic effect of the activator stimulates its production. Such multiple peaks in one dimension, and the corresponding formation of spots (isolated peaks) or stripes in two dimensions, provides a model for the formation of repeated structures with more or less regular spacing (see the discussion in appendix A.4).

Initiation of Regular Patterns in One Dimension The distribution of the multiple peaks depends on the mode of initiation of the pattern [112]. In a *growing domain*, for example in one dimension, initially a monotonic gradient is formed. As the size of the system becomes large enough to accommodate a second maximum (corresponding to a full chemical wave fitting into the system) the secondary peak of activation is formed at a fairly well-defined position, beyond the range of inhibition from the previous peak. Further growth leads to the formation of multiple peaks, with a regular spacing between them, as each successive peak is initiated just beyond the position at which the inhibitor distribution from the nearest active centre has fallen below some critical level. A similar regular peak spacing is obtained if pattern formation is initiated at one end of the domain, by a local increase (possibly externally imposed) in the concentration of activator or inhibitor. The growth of a one-dimensional region with multiple peaks may also lead to the generation of new, intercalary structures; once two activating centres become sufficiently separated, a third peak might form at an optimal intermediate position.

The formation of such regular multiple peaks has been proposed as a model for *phyllotaxis*, the regular arrangement of leaves on the stem of a plant. The model proposes, in the light of experiments involving surgical intervention or treatment with plant hormones, that each new leaf primordium forms where the total inhibitory influence arising from the distribution of inhibitor about existing primordia is a minimum [229]; a regular spacing then results, by the above mechanism, either as a result of apical or intercalary growth. The computer simulations were performed on an elongated cylinder; depending on parameter values, alternate, opposite or parallel arrangements of activator peaks which can initiate leaf formation were formed; this

corresponds to the diversity of observed leaf patterns.

A similar simulation of the initiation of secondary centres of activation on the surface of a growing cylinder, has been used to account for the budding of hydra. This is based on the activator-inhibitor model for hydra described in section 4.2.2: the creation of buds arises from growth beyond a critical length, at which point the formation of a new head is stimulated through a local peak in activation. Eventually the secondary head structure separates from the parent hydra and regenerates fully to form a new complete organism.

Irregular Two-Dimensional Patterns In a sufficiently large two-dimensional domain, multiple peaks of activation lead to 'bristle-like' patterns [234]. Again in this case, fairly regular spacing may be achieved if peaks of activity are generated in sequence, for instance by initiation at a margin, or marginal growth. More likely, however, depending on the mode of initiation, is a more or less *irregular* pattern, which results if the generation of local maxima of activation is *initiated by random fluctuations*, after the system has reached a sufficiently large size for multiple peaks. These peaks, though irregularly spaced, are distributed according to second-order statistics, as the effect of inhibitory fields around peaks of activation causes both small and large distances between peaks to be avoided. Random initiation may in fact result in some local maxima initially appearing too close to one another, since the inhibition originating from the incipient centres is too small; but with increasing activator concentration, the mutual inhibition also increases and, therefore, some of the initially present activator peaks are, in the course of time, suppressed. An irregular distribution thus arises, but a maximum and minimum spacing are nevertheless observed.

Such irregular patterns are observed in nature for example in the distribution of stomata on plant leaves, the spacing of cilia on the epidermis of *Xenopus* embryos, and the bristles and hairs on some bugs [229]. For stomata, for example, experimental measurements indicate that the details of the pattern on each leaf are different, but the general texture of the pattern, based on second-order statistics in the distance distribution, is maintained; furthermore, the formation of new stomata at optimal spacings between the old ones resulting from leaf growth is observed, as predicted by the theory. The activator-inhibitor mechanism has thus been able at least to *simulate*, if not to account for in mechanistic and molecular detail, the formation of some irregular surface patterns in nature.

Analytical Treatment of Multiple Peaks As the formation of multiple peaks corresponds to the behaviour of the reaction-diffusion system some distance from bifurcation (if for example the size of the system is treated as the bifurcation parameter), the analytical treatment of this situation is considerable more difficult that for the one-dimensional formation of gradients. An analytical treatment, using the methods of synergetics, of the generation of multiple-peak patterns has been given by Haken and Olbrich [143]; in two dimensions, for example, regular hexagonal solutions could be found. The question of stability of such solutions is not fully resolved, and can best be studied using computer simulations. We should however note that for problems of biological development, we do not necessarily require absolute stability over infinite time scales, just quasi-stability of a pattern for a period sufficiently large (by some appropriate factor) compared to the time required for the laying down of a biological structure [112].

Stripe Formation

Further models proposed by Meinhardt and Gierer have been designed to account for the formation of striped patterns in two dimensions. Such patterns may occur in an elongated, quasi-one-dimensional domain (where the size in one direction is insufficient for multiple peak formation, so that prepatterns form effectively only in the other direction — see appendix A.4), or, for a region with comparable extension in each direction, if the domain is inhomogeneous, for example if the diffusity, and hence the range of the inhibitor is different in the two directions.

A third possibility has, however, also been proposed, and that is through the formulation of models with a specific tendency to generate stripes. One of the reaction-diffusion models which has been advanced to account for repeating patterns depends on two substances whose synthesis is locally mutually exclusive, but which activate one another at a distance; this property is called 'lateral activation' by Meinhardt and Gierer [235]. As usual, they have proposed several slightly different formulations with varying properties; the principle is well-illustrated by a model of the lateral activation of a system consisting of only two elements:

A Lateral Activation Model We assume the existence of two genes, say G_1 and G_2, which produce autocatalytic products with concentrations g_1 and g_2. These products decay into metabolites s_1 and s_2 which are more highly diffusible than the initial gene products, and each of which activates the other gene of the pair. To avoid unbounded growth, there is a common repressor, r, whose synthesis depends on the concentration of both products and which inhibits both genes. The net result is that each gene activates itself at short range but activates the other at long range. This mechanism may be mathematically formulated as follows:

$$\frac{\partial g_1}{\partial t} = \frac{cs_2 g_1^2}{r} - \alpha g_1 + D_g \nabla^2 g_1, \tag{4.18}$$

$$\frac{\partial g_2}{\partial t} = \frac{cs_1 g_2^2}{r} - \alpha g_2 + D_g \nabla^2 g_2, \tag{4.19}$$

$$\frac{\partial s_1}{\partial t} = \gamma g_1 - \gamma s_1 + D_s \nabla^2 s_1, \tag{4.20}$$

$$\frac{\partial s_2}{\partial t} = \gamma g_2 - \gamma s_2 + D_s \nabla^2 s_2, \tag{4.21}$$

$$\frac{\partial r}{\partial t} = cs_2 g_1^2 + cs_1 g_2^2 - \beta r; \tag{4.22}$$

where c, α, β and γ are constants, and D_g and D_s are the diffusion rates, with $D_s > D_g$. In two dimensions, by contrast to the standard activator-inhibitor model which produces isolated peaks of activation, this model will lead to the formation of long stripes in isotropic tissue, because the two types of zone, with activation of g_1 and g_2, are each dependent on the proximity of the other.

This lateral activation formalism may be extended to more than two activating components; this enables the specification of a sequence of more than two structures, which has been used to account for intercalary regeneration in insects [235]. The detailed pattern of the stripes depends, as usual, on the specific mode of initiation: for example, random perturbation produces an irregular striped pattern, whereas initiation of the pattern by a local disturbance at one end of the field may lead to the generation of semicircular stripes.

The fundamental contribution of Gierer and Meinhardt, to the development of self-organization concepts applicable to biological pattern formation, lay in their recognition of the remarkable pattern-forming properties available through the combination of short-range activation and long-range inhibition. They supported this recognition by demonstrating, with applications, some of the range of patterns achievable through symmetry-breaking and structure formation, both for monotonic gradients and multiple peak patterns. The scope of their proposed applications, however, goes well beyond the initial formation of patterns by a reaction-diffusion mechanism:

A General Theoretical Scheme for Developmental Patterning

Meinhardt, in particular, has used the variety of model systems developed by himself and Gierer in an ambitious attempt to develop a comprehensive and coherent theory of pattern formation during development. These efforts are recounted in his book [229], with more recent developments and results reviewed in [233]. In spite of the titles of these works, — *Models of Biological Pattern Formation* [229] — the book and review article deal virtually exclusively with Gierer and Meinhardt's own models, taking little account of the work of others.

Meinhardt displays an unusually global, holistic perspective: to give a flavour of his breadth of insight, we list some of the isses dealt with:

- The generation of polarity, with applications to slime moulds and hydra;
- Gradients in early insect development;
- Imaginal discs, and compartmentalization;
- Regeneration and pattern regulation in insect legs;
- Pattern formation and regeneration in vertebrate limbs;
- Segmentation and somitogenesis;
- The formation of filament-like structures and branching patterns, with application to leaf venation;
- Pattern formation on the shells of molluscs.

In the main, the models are based on the fundamental 'Gierer-Meinhardt' self-organizing mechanisms for the formation of gradients and periodic patterns, as already discussed, coupled with various deterministic rules and interactions by which the patterns formed at one level cooperate to produce structure at a lower level in the hierarchy. For example, differentiation produces a boundary between different types of cells, which are then assumed, for instance, to produce morphogenetic chemicals interacting at the boundaries to permit the formation of structure at a finer level of detail.

Meinhardt is able to account for a wide variety of experimental observations through suitable combinations of the available pattern-forming schemes, and a continual and welcome stress is laid on the interaction between experiment and theory to build realistic models. Nevertheless, it appeared initially that his work was essentially isolated from the mainstream of theoretical

approaches to developmental biology; this might have been due to its narrow focus, predominantly on the author's own models. Of course, the models described at some length above, have engendered some interest, in particular the fundamental contribution of the role of local activation and long-range inhibition; and more recently, Meinhardt's models for *Drosophila* patterning have attracted attention. Several of Meinhardt's models are discussed in other relevant sections of this thesis (see also sections 4.2.2, 4.2.6, 4.3.2 and 4.3.3).

4.3.2 Integumentary Patterns

We have noted that the regularity, and hence reproducibility, of patterns generated by reaction-diffusion systems, especially in two dimensions, depends on the mode of initiation; patterns can form *sequentially* or *simultaneously*. Those which form sequentially, that is, by commencing in a particular region, and spreading laterally in a wave, for example as a result of growth of the domain, tend to be more evenly spaced and reproducible than those that form simultaneously over the entire domain. When complex patterns form over a large field, as a result of random perturbations, the overall features, such as the tendency towards spots or stripes and the minimum spacing, depend on the kinetics, but the precise details depend on the particular initial conditions for that particular situation. In developing systems there are always inherent stochastic effects, so that patterns formed simultaneously manifest 'stochastic indeterminacy' and can never be exactly predicted from the starting conditions [159].

Bard and Lauder [19] noted this situation, and concluded that Turing's theory was adequate for specifying patterns which need not be well specified — they suggested integumentary patterns such as animal coat patterns, sea shell markings and the distribution of hairs on the skin — but was inadequate if regulation was important. The issue of regulation will be considered separately (see section 4.3.5), but for coat patterns, for instance, the variability in the pattern from one animal to the next is a positive feature of a mechanism. With this in mind, several applications of reaction-diffusion theory to coat patterns and other integumentary markings have been proposed.

Animal Coat Patterns — the Work of Murray

The most prominent of these applications is the classic work of Murray on animal coat patterns [246, 247, 248] (for an elementary introduction see [250]), which we discuss here at some length, as it has become quite well known. He showed, by numerical simulation, that appropriate reaction-diffusion kinetics could give rise to stable patterns *qualitatively* similar to those found in the spot and stripe markings of many mammals. In the light of the striking similarity of the patterns he generated to many of those observed in nature, he suggested that a single simple mechanism might be responsible for producing the enormous variety of coat colour patterns observed; this would have considerable evolutionary advantages compared to any considerably more complex mechanism that required the specification of individual details of any pattern.

The nature of the study was that of a plausibility argument: that the considerable correspondence between the patterns generated by the theory and those observed provided strong circumstantial evidence for the involvement of a simple self-organizing mechanism such as reaction-diffusion in patterning. The details of melanogenesis, the synthesis and deposition of melanin granules, which are responsible for the dark pigmentation of mammalian skin and fur, are still

very incompletely understood; so no attempt was made to model the actual kinetics. Rather, the proposed mechanism was entirely hypothetical, based on, and attempting to provide qualitative support for, the assumption of some simple 'master plan' able to generate a limitless variety of pattern.

Model Mechanism and Equations The proposed mechanism for melanogenesis involves the generation of a chemical prepattern, which is laid down at some early stage of development (in [248] bounds on the time of prepattern formation, based on foetal growth rates, are estimated), well before the pattern becomes apparent. The subsequent differentiation of the cells to produce melanin then simply reflects that spatial pattern of morphogen concentration, and is not treated in the theory. As the detailed mechanism for melanogenesis is unknown, the reaction-diffusion system used for the simulations was chosen purely for illustrative purposes. The practical substrate-inhibition mechanism studied by Thomas and coworkers, based on an immobilized enzyme reaction (described in section 3.2.3; for a detailed discussion of this reaction, see [188]) was chosen, with a plausibility argument presented in support of this choice: the precursor tyrosine, which combines with the melanocytes to produce melanin, is involved in melanogenesis; critical to this process is the presence of the enzyme tyrosinase. Thus it is proposed (purely hypothetically) that the undoubtedly complex multi-species mechanism involved in the melanogenesis reduces to a two-species substrate inhibition mechanism, for S and A, say, such that one of the species could be associated for example with the critical tyrosinase or the tyrosine.

The kinetic equations for this system, with the cosubstrate A diffusing more rapidly than the substrate S, are given above (see equations (3.15)–(3.17)); they are

$$\frac{\partial s}{\partial t} = \gamma f(s,a) + \nabla^2 s, \tag{4.23}$$

$$\frac{\partial a}{\partial t} = \gamma g(s,a) + d\nabla^2 a, \tag{4.24}$$

$$\text{where} \quad f(s,a) = s_0 - s - \rho F(s,a), \tag{4.25}$$

$$g(s,a) = \alpha(a_0 - a) - \rho F(s,a), \tag{4.26}$$

$$\text{and} \quad F(s,a) = \frac{sa}{1 + s + Ks^2}. \tag{4.27}$$

In contrast to the equations in section 3.2.3, we have introduced γ by the choice of a different nondimensionalization for the space and time variables; this is to correspond with the general analysis given in appendices A.2 and A.4. We assume that the reactions and diffusion occur on a two-dimensional domain corresponding to the shape of the integument at the time the pattern is laid down. From our extensive discussions of the possible solution behaviours of reaction-diffusion systems, we know that under appropriate conditions, the above system displays spatially patterned inhomogeneous solutions, which are assumed to be the prepatterns for the coat markings.

The above scheme may be interpreted as a possible mechanism for the development of the patterns [87]: the existence of a substance is postulated, which at high concentrations inhibits melanogenesis early in embryonic development. At later stages, a decrease in the level of this inhibitor is supposed to occur; at this point, fluctuations will trigger diffusive instability, leading to the desired spatial patterns. The patterning process ceases if there is a further decrease

in inhibition, so that diffusive instability will no longer be possible. The result is a spatially inhomogeneous prepattern in the concentration of the morphogens. It is assumed then, that a (genetically determined) threshold concentration exists, such that cells differentiate to produce melanin if the morphogen concentration is above (or below) that threshold. Variations in the level of the threshold will produce a change in the size of the pigmented regions.

The Effects of Scale and Geometry The above kinetics and mechanism, being purely hypothetical, were solely for illustrative purposes, and to motivate the study. The significant part of the analysis [247, 248] was the demonstration of the critical dependence of the final pattern on geometry and scale, which led to a number of associated predictions for the types of patterns which might be observed. A fairly extensive discussion of the effects on patterns of the size of the domain is given in appendix A.4, some of which is recapitulated here; for the analysis, growth in the domain can conveniently be depicted by variation in the parameter γ. We note in particular the intrinsic chemical wavelength of the system, which determines the characteristic size of the pattern features and the number of peaks that appear in each direction of the domain.

If the domain is too small, no structure can form at all, so that uniform markings result. With a larger domain, as γ increases, there is a succession of excitable modes, as the dispersion relation encompasses more linearly unstable modes, and thus the pattern becomes more complex, with the exact pattern features depending closely on the geometry of the domain. For instance, if one of the dimensions is sufficiently small compared to the other, there is a tendency for a succession of modes with non-zero wave number to appear in one direction before the other, so that the first non-trivial multiple peak patterns are quasi-one-dimensional, or striped. That is, stripes predominate in a narrow domain, while spots are more characteristic of wider regions in which structure formation can occur in both directions.

Numerical simulations (using a finite element method originally developed by Kernevez and coworkers [190]) were performed on a domain intended as an illustrative caricature of the shape of the integument at the time of pattern formation, using random perturbations from the spatially homogeneous steady state as initial conditions, and calculated for a range of values of γ, representing the size. The results obtained for successively larger domains indicate that the pattern initially becomes increasingly complex, from a uniform pattern for a small domain to a distribution of spots reminiscent of, say, a leopard coat for an intermediate value of γ. The exact shapes obtained cannot be predicted from linear theory (even for rectangular domains) as the nonlinear effects become progressively more important, and there is interaction between the growing modes. For very large domains, however, the pattern effectively disappears, and the patterning becomes uniform again. This seems reasonable if one notes that the boundaries, which determine the eigenfunctions and hence effectively the structure, play an increasingly smaller role for large regions as most of the reaction-diffusion interactions occur well away from the boundary; alternatively, one might consider that there is insufficient time for pattern formation in a large region, especially considering that the pattern must be laid down in finite time. The numerical results accord well with the observed features of most mammalian coat patterns, in that very small mammals, such as mice, and large ones, such as elephants, tend to be uniform in colour, whereas animals of intermediate size, such as large cats and zebras, are frequently patterned.

Mammalian Tail Patterns — a Developmental Constraint The most striking and ingenious prediction of the modelling relates to the above-mentioned fact that a narrower domain tends to contain striped patterns, being too narrow to sustain genuine two-dimensional patterns, while wider domains may contain spotted patterns. This accords with the tendency for many spotted animals to be striped at their slender extremities, for example the legs. In particular, patterning on the tail may be modelled by treating the tail as a tapering cylinder, and either using cylindrical coordinates with a decreasing radius, or opening the cone by a longitudinal bisection, and obtaining a roughly triangular, tapering, domain, with a narrow end and a broad base, and applying periodic boundary conditions.

The prediction of linear theory in this case, which is borne out by the numerical results, is that the broader part (proximal to the animal) may be thick enough to form spots, whereas the thinner distal part may only sustain stripes. Of course, where the circumference remains small along the length of the tail, then stripes may occur along the entire tail, such as for the genet. The transition of spots at the wide end to stripes at the narrow tip of the tail is thus consistent with the theory, and is frequently observed in nature, such as for leopard and cheetah tails. On the other hand, the opposite transition, from stripes at the broad end to spots at the narrow end, would be inconsistent with the predictions of reaction-diffusion theory. Murray suggests that this is a genuine *developmental constraint* — that whereas a spotted animal may have a striped tail, a striped animal can never have a spotted tail. To date no possessors of inconsistent tails have reportedly been sighted. (For an introduction to Murray's model, see [87], and for a more detailed discussion, see [251].)

Assessment of this Model The wide variety of patterns which could be generated by this simple two-variable reaction-diffusion mechanism, just by varying the single domain size parameter γ, and which have a remarkable qualitative similarity to observed coat patterns, lends credence to the hypothesis [248] that a simple, and possibly universal, self-organizing mechanism based on diffusive-driven instability may be responsible for the generation of mammalian coat patterns. It will probably be long before a detailed knowledge of the mechanism of melanogenesis allows the modelling of its kinetics, and thus the direct verification or disproof of the reaction-diffusion basis of the pattern formation; undoubtedly, the actual mechanism is much more complex, so that this model constitutes at best a considerable simplification or caricature of the most significant aspects. Nevertheless, until more detailed models exist, Murray's model stands as a quite convincing, though totally hypothetical, 'explanation' for the observed patterns.

Most significant is the emphasis and clarification of the critical role of the geometry and size of the integument at the time the pattern is laid down, with the 'free' corollary of the non-trivial prediction of a developmental constraint that has not yet been falsified. It seems likely, however, that similar patterns and associated predictions would follow from any self-organizing scheme with analogous properties of lateral inhibition and a similar kinetic basis (see section 5.1.1); so that what has really been shown is that the observed patterns are consistent with a general *lateral inhibition* class of models, rather than being intrinsically tied to any *particular* modelling scheme, such as reaction-diffusion systems [287].

Various other models attempting to account for coat and wing patterns have been proposed, based on the ability of reaction-diffusion mechanisms to simulate the observed patterns. These

will only be considered here briefly, in the light of the fairly extensive discussion given to the Murray model above.

An Alternative Reaction-Diffusion Model for Coat Patterns

A different model, which addressed itself to similar questions to that of Murray, was proposed slightly later by Bard [14]. He also considered a reaction-diffusion system, and its capability to generate chemical maps whose concentration contours are similar to those observed on the flanks of zebras, cats and other mammals. The specific kinetics he employed were a nonlinear version of the kinetics proposed by Turing [354], combined with the assumption of discrete diffusion between adjacent cells. Numerical simulations on a cellular array were performed for a range of initiating and boundary conditions, and numerous pattern features, including spots and stripes, were obtained.

In contrast to Murray [248], Bard concentrated more on the effects of different modes of initiation; these included uniform, simultaneous activation over a rectangular array; a line of cells that synthesizes an activator diffusing ventrally, and initiates the kinetics in a cell once its concentration there exceeds some threshold — this can give rise to dorsal stripes that break up ventrally into spots — and lastly, initiation through an activator of the Turing kinetics which moves ventrally through the array at constant velocity, which can lead to vertical stripes and the possibility of an additional dorsal stripe. The role of different thresholds for initiation was also considered. Many results similar to those found by Murray [248] were obtained, such as the random variability of pattern details, and the effect of scale and boundary conditions for tail patterns; as well as some additional pattern features that did not appear in Murray's simulations, such as a dorsal stripe and complex spots typical of leopards. The use of a variety of different initiation conditions, while producing a greater range of patterns and lending more generality to the study, appears however to deprive the results of Bard of the elegant simplicity and universality that Murray claims for his theory.

Butterfly Wing Patterns

Diffusion-Based Models Murray has also proposed a model, or rather series of models, for generating various pattern elements common in butterfly wing patterns [247] (for a comprehensive, more recent discussion, see [251]). The diversity of such patterns can be shown to be composed of a relatively small set of basic pattern elements, with the most common being denoted central symmetry patterns, dependent patterns and eyespot patterns. For each of these three cases a slightly different model, depending in particular on geometry, is proposed. The basis of the simple model involves a morphogen, released from pre-existing sources, which operates a plausible biochemical switch mechanism, such as that discussed above [205] (see section 4.2.3) for the interpretation of thresholds to produce a steady-state spatial distribution in a gene product or colour-specific enzyme. This spatial pattern is then reflected in the ultimate pigmented pattern, as pigment cells react to the product level laid down. The computed solutions from the model compare well, qualitatively, with observed patterns on butterfly wings, which provides some support for the diffusion control of pattern. Whereas in general developmental fields pattern is laid down over a maximum dimension of about a millimetre [375], here dimensions of several millimetres are experimentally observed. Nevertheless, diffusion is still a plausible

mechanism, as the pattern is laid down over several days during which the scale and geometry do not vary much; and it appears, in fact, that reaction-diffusion is too rapid a mechanism to accord with observations on the laying down of butterfly and moth wing patterns.

Another study of butterfly wing patterns based on pure diffusion has been performed by Bard and French [18]. They studied the simple diffusion of a *single* morphogen, with pre-defined sources in the foci of eyespots and sinks in the wing margin, and with the overt pattern arising from the interpretation of the local morphogen concentration by cells, with different thresholds corresponding to different colours. Although they found stable patterns having the essential topology of some common features of butterfly wing patterns, they concluded that diffusion of a single morphogen is too limited to generate the full range of complex and reproducible wing patterns observed.

A Comprehensive Model for Butterfly Wing Patterns The two diffusion-based studies discussed above do not strictly fall into the class of self-organizing mechanisms, as the distribution of the morphogen sources, which plays a determinative role in the final pattern, is assumed to be specified *a priori*. A more recent model, proposed by Nijhout [279], attempts to overcome the limitations of the need for pre-existing sources and the inability of a single morphogen to produce a complex pattern. On the basis of computer simulations, he shows that a two-step model is able to generate nearly the entire diversity of colour patterns seen in the butterfly family Nymphalidae. The first step involves the specification of the locations of the sources and sinks of two morphogens, using a self-organizing reaction-diffusion scheme such as the Gierer-Meinhardt activator-inhibitor model. By such a mechanism, a distribution of sources and sinks is created which acts as a 'toolbox', shown by the simulations to be adequate to generate the desired patterns. In particular, two gradients are established by simple diffusion in the second step, and a threshold interpretive scheme responding to the sum of the gradients is postulated. A feature of this work is the extensive comparison with experimental observations, which *inter alia* appear to require a two-step patterning process and long-range signalling.

The Generation and Distribution of Skin Organ Primordia

A novel application of reaction-diffusion theory, which attempts to treat a more detailed situation and thus appears somewhat more speculative, is that by Nagorcka and Mooney on the formation of hair follicles and hair fibres, as well as on the distribution of skin organ primordia for the spacing of mammalian hair [241, 262, 266, 267] (reviewed in [264]). The model is motivated by the practical goal of understanding the developmental and physiological processes underlying the correlations between fibre and follicle characteristics such as fibre diameter, follicle density and fibre length growth rate, with the ultimate aim of proposing new criteria for the selection of more efficient wool-producing sheep. In pursuit of this goal, a reaction-diffusion mechanism is proposed, which under different specifically chosen conditions is shown to display features consistent with the observed spatial pattern for cell differentiation during hair follicle initiation, hair follicle development and hair fibre formation.

For details of the somewhat intricate proposed scheme, the original papers should be consulted. In brief, a reaction-diffusion system involving the interaction between two components X and Y, as proposed by Kauffman *et al.* [180] (see section 4.3.3), is assumed to operate in

two dimensions, with a third substance Z diffusing radially outwards from the dermal papilla. The spatial pattern of the differentiation between different types of fibre cells, and the cells which will contribute to the inner and outer root sheaths surrounding the fibre, is assumed to be determined by the product of the concentrations XZ (it is not made at all clear how a biochemical threshold response can be based on a *product* of concentrations). In this way an isomorphic prepattern in three dimensions is generated, which is proposed to account for hair fibre formation [266]. Positional information provided by the same reaction-diffusion system is also proposed to account for hair follicle initiation [267] and follicle development [241], with the reaction-diffusion system solved separately in the epidermis and within the follicle. Lastly, but under slightly different conditions (assuming the existence of a dermal signal diffusing to the epidermis and triggering the reaction-diffusion system in that region) the generation of a prepattern for the distribution of primordia for hair follicles, with possible extension to feather follicles and reptilian scales, is simulated [262].

Assessment of the Nagorcka-Mooney Model As already indicated, the significance of this work is not quite clear; it has not at all been widely cited in the rest of the literature. It has the strength of being very closely linked to experimentation, and a constant emphasis is made on the need for correspondence between the model predictions and observations. This also follows from the unusual economically-motivated nature of the project. However, the current state of theoretical approaches to development is not really sufficiently far advanced to handle such a complex situation; at present one is more concerned with exploring what is *possible* with self-organizing mechanisms such as reaction-diffusion. Thus the authors find themselves being forced to introduce a multitude of seemingly *ad hoc* assumptions and apparently arbitrary rules, beginning with a reaction-diffusion system without mechanistic or experimental motivation, in order to generate the desired sequence of patterns. It appears that the models [241, 262, 266, 267] may thus not have much *explanatory*, as opposed to descriptive, value: The generated patterns seem only to correspond with the assumptions put into the model, chosen to produce just the desired sequence; so no necessarily new insights seem to be available.

Nevertheless, the work is notable for its ambitiousness, and especially for the emphasis laid on the possibility for the formation of a *sequence* of prepatterns, coupled in a *hierarchical* way. Such a sequence of changing patterns may occur when a previously laid down pattern instigates a change in the geometry or size of the tissue containing the morphogens, or alternatively a variation in the diffusion and/or reaction rates of the morphogens. A change in the parameters or boundary conditions can significantly affect the produced pattern, as discussed at some length in appendix A.4, so that one pattern can act as a template for the generation of the next pattern under different conditions — there is *feedback control* of the pattern formation mechanism onto itself, permitting a continuously changing prepattern. Such a concept of the formation of a sequence of isomorphic prepatterns was well illustrated in the descriptions of sequential pattern formation at different stages in the growth of follicles [241], as well as the successive waves of primary follicle initiation [267].

Mollusc Shell Patterns

The final example of the application of reaction-diffusion theory to integumentary patterns is in the simulation of pigmentation and relief patterns on the shells of molluscs [236]. Shell pigmen-

tation patterns, even more than other integumentary patterns, show an enormous variability, even within species, and do not appear to have much selective significance, especially as in many cases the animals live burrowed in the sand or are active only at night. This presumably facilitates the generation of diversity, and supports the assumption of a pattern-forming mechanism such as reaction-diffusion, whose stochastic indeterminacy permits the creation of a range of patterns with the same mechanism [236]. A variety of models to account for pigmentation patterns has in fact been proposed, and we shall encounter neural models [96] and cellular automaton simulations [359] for such patterns later (see sections 5.1.4 and 6.2.2, respectively).

A Reaction-Diffusion Mechanism — Meinhardt and Klingler The pigment and relief patterns on shells are formed via secretions from cells constituting the mantle edge of the mollusc shells. In the light of this, Meinhardt and Klingler [236] have suggested that the mantle edge may be regarded as a one-dimensional domain containing a reaction-diffusion-based prepattern mechanism, and that the resultant shell pattern is simply a time record of what has happened at the growing edge during the life span of the animal. Hence the two-dimensional pattern is of the nature of a *space-time plot* of a possibly slowly changing prepattern mechanism operating in one dimension.

The assumptions of the model were, as usual, that within each cell an autocatalytically produced 'activator', a, stimulates pigment secretion, and that cells communicate with their neighbours by diffusion. The inhibitory effect on the activator production was modelled either by the depletion of a substrate, s, which could be a precursor necessary for activator production, or by the effect of an inhibitor, h, whose production was catalyzed by the activator. These schemes were formulated, respectively, by

$$\frac{\partial a}{\partial t} = \rho s \hat{a}^2 - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \tag{4.28}$$

$$\frac{\partial s}{\partial t} = \sigma - \rho s \hat{a}^2 - \nu s + D_s \frac{\partial^2 s}{\partial x^2}, \tag{4.29}$$

for the activator-substrate interaction, and

$$\frac{\partial a}{\partial t} = \frac{\rho(\hat{a}^2 + \rho_0)}{h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}, \tag{4.30}$$

$$\frac{\partial h}{\partial t} = \rho \hat{a}^2 - \nu h + \rho_1 + D_h \frac{\partial^2 h}{\partial x^2}, \tag{4.31}$$

for the kinetics between activator and inhibitor. In both cases the saturation of the activator is modelled by

$$\hat{a}^2 = \frac{a^2}{1 + \kappa a^2} + \rho_0 \tag{4.32}$$

(ρ_0, the activator-independent activator production, was neglected in the activator-inhibitor model), and all symbols except a, s and h are constants.

A range of simulations was performed using the above kinetics, with minor modifications being employed to mimic certain specialized shell patterns; several of the computer simulations, and the listing of a program used for the simulations, are given in [237]. A wide range of patterns reminiscent of those observed on shells was obtained when the simulation conditions were varied

for each situation, with stripes parallel and perpendicular to the growing edge forming the simplest cases. More intricate patterns exploited the oscillatory nature of the kinetics, and postulated a time delay for activation of successive cells, such that travelling waves could exist; these generated oblique lines and crossings, with branching patterns indicating the interaction between waves and the sudden formation of backwards waves. The superposition of spatially stable and periodic patterns, or two time-dependent patterns, was able to produce yet more detailed shapes, as was differential growth along the domain, generating spiral shells such as *Nautilus*.

By appropriate modifications of the reaction kinetics and boundary conditions, a wide variety of patterns strongly resembling observed structures was generated — for details and results of the simulations, see [237, 236], or for an introduction the review in [233]. This provided circumstantial support for the notion that a simple and universal mechanism such as reaction-diffusion might be responsible for the shell patterns. The similarity of these simulated patterns to those produced, for example, by neural models [96] (see section 5.1.4), however, indicates that it is the underlying self-organizing feature of local activation and lateral inhibition that is ultimately responsible for the striking pattern-forming features, rather than the precise details of any mechanism such as reaction-diffusion *per se* [287, 288].

4.3.3 *Drosophila* Segmentation and Compartmentalization

In the context of repeated patterns in development, a natural central role is played by the study of the segmentation of the fruit fly, *Drosophila melanogaster*. A broad outline of the early development of *Drosophila* is given in section 2.2.3, together with a description of the hierarchical sequence of gene expression which progressively subdivides the embryo. By the time the original syncytium is subdivided by cell walls, the blastoderm is divided into seven (and later fourteen) segments, each of which will follow an autonomous developmental pathway determined by the particular combination of homeotic genes expressed in that segment. The prospective segmentation pattern is already apparent in the banded expression patterns of the segmentation genes well before cellularization, which exhibit a definite *temporal sequence of striped expression*.

The time evolution of some of the mRNA and protein products of the segmentation genes has been well characterized by *in situ* staining techniques, using monoclonal antibodies to detect protein concentrations, or mRNA probes consisting of radioactively labelled cDNA. The extent of knowledge about the hierarchical control of the expression of many of these genes, together with the fairly detailed understanding of their structures, activation and properties (see for example [77, 200]), indicates that a molecular-kinetic model of the expression of the segmentation genes, based on the known molecular interactions, may be feasible in the near future. The current state of knowledge is however not that far advanced, and the mechanism is bound to be intricate, so it seems reasonable here, as elsewhere, to attempt first to seek understanding through the use of simpler, if not necessarily realistically-based, models.

The observed spatially periodic concentration patterns are immediately suggestive of some underlying mechanism that tends to produce *periodic wave-like patterns*, such as a reaction-diffusion mechanism or analogous scheme. The proposed mechanism would have to explain the observed sequence of expression patterns, eventually to yield seven stripes, and especially to

account for the maintenance of the number of metameres (segments or parasegments) in the face of variations in the length of the embryo; as well as, if possible, provide some form of explanation for the modification or deletions in particular stripes obtained through mutations in the relevant genes. Several attempts have been made to account for *Drosophila* segmentation on the basis of symmetry-breaking reaction-diffusion mechanisms.

Drosophila — **a Challenge for Kinetic Modelling** The extensive experimental evidence on *Drosophila* gene expression patterns has led reaction-diffusion modelling to enter a totally new phase, and required a serious re-evaluation of the status of these models. No longer do theorists have the 'luxury' of being able to generate patterns that simulate observed structures without having to submit them to the rigours of experimental verification. Rather, the possibility exists for the first time that the patterning theories may be tested against the known molecular properties and dynamical behaviours of the gene expression patterns.

In consequence, the position of reaction-diffusion models here is not quite clear: On the one hand, it has been suggested that the recent successes of reaction-diffusion systems at producing sequences of striped patterns have reawakened interest in the concept of prepatterns and reaction-diffusion models *vis-á-vis* embryonic body segmentation, so that at last experimental support for such models may be forthcoming [159]. On the other hand, we have seen that the *Drosophila* segments are each independently specified by a particular combination of egg-polarity, gap and pair-rule genes (see section 2.2.3), so that the superficial symmetry and periodicity appears to conceal an underlying fundamentally *aperiodic* structure, thus specifically ruling out reaction-diffusion mechanisms producing repeating patterns [327]. For now, we take note of this tension, which can only be resolved through further focussed modelling and experimentation, and proceed to describe some of the models that have been proposed for *Drosophila* patterning.

Sequential Compartmentalization — the Model of Kauffman *et al.*

The earliest, and possibly the most influential, attempt at kinetic modelling of *Drosophila* was made by Kauffman, Shymko and Trabert in the late 1970s [180]; this constituted one of the first serious attempts to apply reaction-diffusion theory to development. This work occurred before the detailed knowledge of gene expression patterns described above was available (so that the cautionary comments of the preceding paragraphs do not apply here), and devoted itself to an account of the sequential commitment to alternative developmental programmes that occurs in neighbouring groups of cells. This commitment appears to be reflected by lines of clonal restriction, called compartmental boundaries, which progressively subdivide the early embryo, on a hierarchical basis; such that cells in a particular compartment are committed to a certain developmental pathway [70] (see section 2.2.3).

The suggestion of Kauffman *et al.* [180] was that a reaction-diffusion system, in a growing domain, generates a sequence of differently shaped chemical patterns, whose nodal lines (of zero concentration) are assumed to correspond to the compartmental boundaries. In response to each pattern, a binary 'choice' is made, which determines a region in the blastoderm (or a cell, after subdivision of the syncytium through the formation of cell walls); once the heritable determination of a region has been achieved, the responsible prepattern is superseded by another.

In this way each region, or terminal compartment, acquires a unique binary 'code', reflecting the sequence of prior determinations, which uniquely specifies its later developmental pathway [178].

The predicted compartmental boundaries correspond to the nodal lines of the reaction-diffusion system on the domain, which was approximated by an ellipse. We already know that the spatially heterogeneous solutions to a reaction-diffusion system to first order correspond to the eigenfunctions of the Laplacian operator on the domain of interest (see appendix A.2). As this result is obtainable also from linear theory, Kauffman *et al.* considered mostly a generic linearized reaction-diffusion system for computational simplicity; on an elliptical domain with no-flux boundary conditions, the Laplacian eigenfunctions are then Mathieu functions [180]. From the sequence of successive nodal lines of the eigenfunctions corresponding to increasing eigenvalues, a predicted binary combinatorial code for each compartment was calculated, and the effect of modifications in single positions of this code compared with the directions of *cross-compartmental* changes observed in transdetermination and homeotic mutations, with considerable (albeit not complete) success. The features of this work were intended to emphasize the possible role of the reaction-diffusion system in *Drosophila* patterning, before the days of the explosion of interest in segmentation and stripe formation, with particular reference to the likelihood of a sequence of binary commitments (corresponding to successive positional information) to locate and determine a cell or region in the embryo — see [178] for a discussion of the combinatorial epigenetic code.

Compartmentalization in Imaginal Discs The most significant aspects of the study of Kauffman and coworkers were not, however, related so much to early compartmentalization as to the formation of lines of clonal restriction in the *imaginal discs* of *Drosophila*. In the larval tissues, there are specific groups of cells, called imaginal discs, which will undergo growth and differentiation at the final metamorphosis to produce adult structures such as eyes, legs and wings. Each of the imaginal discs, in turn, may further be subdivided into groups of cells destined for specific parts of the final structure, and there are specific boundaries, subdividing for example the wing disc, such that if a cell is marked early in development, its progeny — a clone — keep strictly within the compartmental boundaries (see section 2.2.3; for an early introduction to the results of clonal analysis, see [70]). Furthermore, by similar clonal techniques it is possible to ascertain the sequence of compartmentalizations appearing spontaneously as the imaginal disc grows.

The ingenious suggestion of Kauffman *et al.* [180] was based on the fact that due to the intrinsic chemical wavelength of a reaction-diffusion system, growth of the system results in the excitation of successively higher modes, as more and more waves can fit into the domain (see appendix A.4). Thus they proposed that in the growing imaginal disc, a discrete sequence of chemical wave patterns is created, each pattern decaying to make way for the next. As noted by Lacalli and Harrison [197], such behaviour, with the pattern markedly unstable to increase in size, requires specific nonlinear kinetics; Brusselator-type models are more suited to such successive patterning than the fairly stable Gierer-Meinhardt kinetics [150] (the nonlinear equations proposed in [180] and used for numerical simulations do satisfy the requirement of qualitative instability). The nodal lines obtained successively on an ellipse correspond well with the observed time sequence and geometry of compartmentalization. This result was very suggestive of the conclusion that the sequential process of compartmentalization could arise ***spontaneously***,

solely due to the succession of self-organizing modes that can appear through increasing domain size, pointing to the possible involvement of some analogous kinetic mechanism in compartmentalization (see also the discussion in [87]).

Critique of the Compartmentalization Model The results obtained by Kauffman *et al.* [180] have been questioned by Bunow and coworkers [47], who performed numerical simulations of the full nonlinear system on a more realistic domain. They showed that the pattern of nodal lines of successive eigenfunctions was very sensitive to the shape of the domain; the use of a more realistic shape for the wing disc yielded nodal lines that differed significantly, both in form and order, from those obtained for an ellipse, and also from those observed on the wing disc. Similar difficulties were found for the predictions for compartmentalization in the early embryo, where discrepancies were again found when calculations were done on the surface of an ellipsoid, a more realistic domain shape for the blastoderm than an ellipse.

The cause of the difficulty was the fact that Kauffman and coworkers [180] used a linear model to determine the nodal lines from the eigenfunctions, which was unrealistic and ultimately unstable. On the other hand, for any nonlinear model, *a priori* prediction of the sequence of patterns resulting for given parameters is impossible, that is, the linear predictions do not necessarily compare with the numerical calculations. The patterns generated by nonlinear reaction-diffusion models are generally quite sensitive to changes in the shape of the domain and physical-chemical parameter values; the conclusion of Bunow *et al.* [47] was that, in the face of such sensitivity, it was difficult to see how the strictly reproducible sequence of patterns could be maintained subject to normal biological variation and perturbations. Thus it appeared that the suggestion that the sequence of compartmental lines could be generated by successive eigenfunctions of a reaction-diffusion mechanism, while ingenious, was ultimately not stable enough to be plausible.

Segmentation and Stripe Formation in *Drosophila*

The model of Kauffman *et al.* [180], in addition to being problematic, is today largely of historical interest. In the intervening years, the surge of enthusiasm for and knowledge about *Drosophila* genetics has caused attention to be directed at gene expression patterns; compartmentalization is less fundamental, being essentially a consequence, or epiphenomenon, of these patterns, and is thus of diminished interest. A natural target for the attentions of reaction-diffusion theorists is the seven-stripe pattern in the *Drosophila* embryo; and indeed, at least three independent attempts at accounting for this pattern have been made. We will refer to them by their originators or principal exponents.

Meinhardt The first is due to Meinhardt (see in particular [232], and the reviews in [229, 233]), on the general approach of combining a number of the mechanisms in the 'Gierer-Meinhardt repertoire', in a suitable order and with appropriate rules for the different stages, to generate the desired pattern. Thus a reaction-diffusion system, probably an activator-inhibitor system (given for example by equations (4.7)–(4.8)), is used to set up a simple gradient of positional information along the anterior-posterior axis. The nuclei are then postulated to be able to read the gradient, through a set of four thresholds which divide the syncytial blastoderm into five 'cardinal regions', each with a specific pattern of gap gene expression, along the anterior-

posterior axis. The border between the cardinal regions acts as an organizing region for the formation of the first periodic patterns. The formation of stripes, to divide the embryo into segments, parasegments and compartments, is assumed to proceed on the basis of a reaction-diffusion mechanism with a tendency towards striping, such as the lateral activation mechanism discussed above (equations (4.18)–(4.22)).

Further detail is established and stabilized by induction between neighbouring cells and compartments, through for example interactions and gradient formation at the borders; depending on the rules used, various patterns and pattern changes result, including frequency doubling, that is, the number of bands doubles in each successive pattern, as observed in the increasingly finely striped embryo. Eventually, the series of hierarchical inductions leads to a periodic pattern of segments due to the periodic repetition of (at least) three distinct cell states [232].

The suggestions of Meinhardt involve a combination of rules and mechanisms utilized in a particular well-defined hierarchical sequence, to produce the observed patterns. As pointed out by Nagorcka [263], such a scheme is quite complex, and lacks the elegant simplicity of the model, say, of Kauffman *et al.* discussed above [180]. On the other hand, there can be no objection in principle to having a variety of different mechanisms in a time sequence, as any information needed to trigger the different schemes can readily be encoded in the genome, and the mechanism switched on in response to a signal arising, say, from the results of the previous stage. A single mechanism, switched on once and left to run its course, would indeed seem to have less error-correcting potential than a hierarchical sequence of schemes, the activation of each stage depending on the successful completion of the previous one. There is sufficient information in the genome to encode and account for the sequential use of different pattern-forming mechanisms; this is still considerably more economical than the individual specification of each position in the embryo.

An earlier, similar attempt at providing a unified view of *Drosophila* development, linking the establishment of the primary anterior-posterior axis, segmentation, compartmentalization, and imaginal disc formation, growth and regeneration, has been made by Deak [76], who combined in particular the models of Meinhardt for the various stages into a coherent formalism. This work does not seem to have received much attention, however.

Harrison, Lacalli and Coworkers Whereas Meinhardt [232] does not explicitly specify the mechanisms involved in gradient formation and induction, except to point out that such processes are feasible given the palette of schemes available, Harrison [156] has studied Meinhardt's scheme to investigate where in the hierarchy of gradient formation/positional information, stripe formation and induction, reaction-diffusion schemes may be operative. He concluded that such self-organizing mechanisms may play a role throughout the pattern-forming process. In particular, he argued that the rules given for inductive processes, including self-activation of genes, mutual interaction including inhibitions, and communication by transport of molecules, were very suggestive of a kinetic mechanism such as reaction-diffusion, and that reaction-diffusion theory can account for the constancy and stabilization of the number of metameres, provided the variation in concentration of precursors to the reaction-diffusion scheme, which may appear in the expression for the chemical wavelength (see for example equation (3.34)), is taken into account [150].

Whereas reaction-diffusion theory is usually discussed in the continuum approximation, with

concentrations a continuous function of space and time, the presence of cell membranes (after cellularization), or of nuclei in the syncytial blastoderm, can introduce a coarse compartmentalization of the diffusion region. In this case discrete diffusion may result, as crucial steps of the chemistry may be located at the nuclei, or cell-to-cell exchange via gap junctions may occur; when this is taken into account in the computations it can amount to the use of a very small number of spatial points per reaction-diffusion wavelength. Harrison and Tan [156] considered this case, and performed computer simulations which indicated that the reaction-diffusion system may be metamorphosed into a switching mechanism, with an on-off pattern which resembles the pattern of expression of some of the segment polarity genes, in particular the gene *engrailed*.

Further computations performed by Harrison, Lacalli and their coworkers have attempted to reproduce the stable pattern of seven stripes in a single mechanism, on the basis of the suggestive similarity of the striped segmentation pattern of *Drosophila* to a reaction-diffusion system. Computations have largely been performed on the Brusselator or minor modifications of the scheme, which has been shown [194] to have greater capacity than the Gierer-Meinhardt model to maintain regular spacing in a pattern of many parts.

The major problems to be considered are the stabilization of the correct *number* of patterns with respect to size variation — for instance through variations in precursor concentrations — and especially the issue of how to induce the reaction-diffusion system to show a preference for stripe rather than spot formation in two dimensions. A review of methods that have been utilized for this purpose exists [211]; the major techniques that have been attempted by Lacalli, Harrison and coworkers include the use of:

1. An anterior-posterior gradient (such as the *bicoid* gradient) which acts as an axial asymmetrizing influence, and is thus able in conjunction with sharp anterior and posterior boundaries to orient and stabilize multiple patterns [198].

2. Reaction-diffusion systems involving four interacting morphogens arranged in symmetrical pairs, such as for the lateral activation model (equations (4.18)–(4.22)) of Meinhardt and Gierer [235]; in particular, it is assumed that the two self-activating morphogens, expressed in complementary out-of-phase stripes, are products of early-acting pair-rule genes, such as the primary pair-rule genes *hairy* and *runt* [195].

3. Models involving special functional forms for the kinetics (such as antisymmetric reaction terms [263] — see below) [211].

Nagorcka A further, similar attempt at providing a sequence of reaction-diffusion patterns that may provide prepatterns for segmentation and compartmentalization, the last to be considered here, has been made by Nagorcka [263] (see the review in [264]). He argues on the basis of numerical simulations that reaction-diffusion kinetics in which the nonlinear reaction terms are *antisymmetric* with respect to the uniform steady state, show a strong tendency towards the formation of striped or banded patterns.

For the *Drosophila* simulations, a reaction-diffusion system with antisymmetric kinetics is solved on the surface of a prolate spheroid, representing the surface of the syncytial blastoderm; and successive solutions are obtained, following every nuclear division cycle (from cycles 8 to 14). The critical assumption made in order to generate the desired sequence of patterns with

frequency-doubling — consistent with the fact that the number of stripes of, say, the *fushi tarazu* gene doubles with each cycle — is that the density of nuclear material, n, plays a crucial role in the system. In particular, increasing density impedes the diffusion of the components, so that D is assumed to be proportional to n^{-1}, while the reactions are assumed to be speeded up at a rate proportional to n. It follows that the characteristic wavelength λ of the system, which measures the balance between reaction and diffusion rates, is proportional to n^{-1} (it is a general result that for general reaction rate constants k and diffusivities D, the chemical wavelength λ is proportional to $\sqrt{D/k}$ — compare the expression for the Brusselator (3.34), if all ks and all Ds are set equal); so that after every synchronous nuclear division cycle, when n doubles, λ halves, accounting for the observed frequency doubling effect. (During the first two cycles considered, 8 and 9, the variations in the pattern are deemed to be due to the growth of the syncytium.) The observed patterns correlate well with the sequence of expression patterns for the genes *fushi tarazu* and *paired*.

The emphasis of this study, as in the other work of Nagorcka and Mooney [264] (see section 4.3.2), is on showing how a *sequence* of isomorphic prepatterns may be produced by a single mechanism, if the geometry or parameters of the reaction-diffusion system change and act as a template for the next iteration, providing a feedback mechanism. Such a mechanism, it is argued, can thus generate sufficient effectively unique spatial information to obviate the need for thresholds or a complex interpretive scheme; although a set of genes to interpret the prepatterns, in this case probably given by the segmentation genes (which in turn regulate the homeotic genes, building up a gene network which can be likened to a series of binary switches — see [178]), is required. Details of Nagorcka's scheme may be found in [263].

Drosophila bears the promise of a detailed understanding of the (possibly self-organizing) kinetics of at least *one* developmental system in the not too distant future; in pursuit of this goal, there is little doubt that the humble fruit fly will remain an organism of major interest, both for genetic analysis and for modelling.

4.3.4 Algae, Limbs, and other Applications of Reaction-Diffusion Mechanisms

A number of other applications of reaction-diffusion theory have been made to aspects of developmental systems, which have however not been so prominent or detailed; some of these will be outlined here briefly.

Modelling hair patterns in a whorl in *Acetabularia*

The green marine alga *Acetabularia mediterranea* is a giant unicellular organism which displays remarkable powers of regeneration, analogously to the multicellular *hydra*. The alga is a narrow stalk, about 4–5 cm long, on top of which is a round cap about 1 cm across. After removal of this cap, apical regeneration proceeds by a series of distinct stages, beginning with the formation of a conical tip which elongates, and then flattens, following which a peripheral ring of hairs, known as a whorl, is formed. Further extension of the stalk can take place with the formation of other whorls; the process can be repeated a number of times depending on growth conditions, before the tip flattens and a cap primordium, considerably more complex than the whorls but with the

same radial symmetry, is formed, and grows eventually to produce the intricately sculpted adult cap [131, 251].

Motivation for a Kinetic Approach Of particular interest in this process is the periodic distribution of the whorl hairs, with a characteristic spacing, which is independent of the size of the tip (at a given temperature); that is, the *number* of hairs varies strongly (between about 5 and 35), but in (approximate) constant proportion to the radius of the stalk. This corresponds well to the predictions of a Turing-type reaction-diffusion theory, in which the number of structures is not scale-invariant, but the patterns display a characteristic 'chemical wavelength' depending on the kinetic parameters and precursor concentrations of the system. The intrinsic scale length between *Acetabularia* hairs is thus evidence in favour of a reaction-diffusion or similar kinetic mechanism for the determination of the whorl hair positions. The temperature dependence of the spacing has been investigated by Harrison and coworkers [155], who noted that the dependence appeared, to within their experimental resolution, to have an Arrhenius dependence like that of a chemical rate parameter — the logarithm of the spacing depended linearly on $1/T$. This provided further motivation to seek a reaction-diffusion-based explanation for the whorl formation, as concluded initially in [155, 194].

A particularly interesting feature of *Acetabularia* regeneration is that it depends crucially on free calcium concentration [152]. There are definite limits to the concentration of Ca^{++} in the external medium within which whorl formation will take place, indicating that calcium is intimately involved in the initiation of a whorl of hairs. Furthermore, the functional form of the dependence of the hair spacing on calcium concentration (a linear dependence on $1/[Ca^{2+}]$) corresponds well with the predictions of some hypothetical reaction-diffusion mechanisms. On this experimental basis, calcium has been proposed as a morphogen in *Acetabularia*, making this one of the few systems where a plausible candidate for morphogen has been identified (see the discussion in [251]). Thus a reaction-diffusion model with calcium as a morphogen was proposed by Harrison and coworkers [155, 154, 152], as discussed above, and another independently by Goodwin and coworkers [129].

Comparison of Models with Experiment The formulation and analyses of these models are not treated here; we note merely that throughout the development of these models, there has been a constant emphasis on the connection with experiment, which was possible here as the quantitative influence of various factors on morphogenesis was known. Thus, for instance, the results of temperature shock experiments, where the alga initially incubated at one temperature were transferred to a water bath with a different temperature at some stage during the regeneration process, were compared by Harrison *et al.* to the predictions of Turing's reaction-diffusion model in terms of the competitive growth and decay of patterns with different spacings at temperature-dependent rates [154].

Similarly, Goodwin *et al.* [129] studied a model (Schnakenberg) reaction-diffusion system in an annulus, corresponding to the domain within which pattern formation takes place, and the variation of the wavelength with calcium concentration was compared with the effect of the variation of one of the parameters (see the discussion in [251]). Further work by Goodwin and coworkers (cited in [131]) presented a more realistic reaction-diffusion model for the kinetics of interaction between cytosolic free calcium and cyclic-AMP, assumed to be the other morphogen

(again on the basis of experimental evidence for the importance of cAMP in cellular metabolism and particularly calcium balance). It was pointed out, however, that the requirement that the inhibitor in a reaction-diffusion system diffuse faster than the activator (in general $d > 1$) posed a problem as it would have required the much larger cAMP molecule to have a higher diffusion coefficient in cytogel than Ca^{2+}. Even this apparent difficulty can be overcome, however, if calcium is assumed to participate not as a morphogen, but in a complex that acts as a morphogen precursor [152], or if the morphogens are considered to be membrane-bound [154].

Acetabularia — a Reaction-Diffusion System? The evidence in favour of a reaction-diffusion mechanism for the patterning of whorls in *Acetabularia* regeneration appears to be quite strong, with considerable experimental support and a plausible candidate for a morphogen known to have a definite influence on the patterning. Nevertheless, not all is cut and dried, for, as we shall see in depth in the next chapter, there are alternative classes of self-organizing models proposed for morphogenesis, based largely on mechanical interactions within and between cells. One of these mechanochemical models, based on the interaction of intracellular calcium (which we know to be significant) with the viscoelastic cell cortex or cytogel, has been proposed for *Acetabularia* and studied in detail [131] (see section 5.2.2). Comparisons of the two approaches have been given in [131, 150], and focussed experimentation is now required to discriminate between the predictions of these models [151]. Nevertheless, this unicellular organism, like *Dictyostelium*, provides a highly useful model system, for the evidence in favour of *some* model displaying symmetry-breaking, self-organizing behaviour does appear to be fairly unequivocal.

Reaction-Diffusion Model for Skeletal Patterning in the Chick Limb

We have already had occasion to consider the important and much-studied problem of skeletal patterning in the developing chick limb in the context of positional information (see sections 2.2.2, 4.2.4), and we will return to it when considering mechanochemical theories of chondrogenesis (the formation of cartilage condensations that instigate the generation of skeletal rudiments) in the next chapter (see section 5.1.1) — for a recent review of experimental and theoretical approaches, see Maini and Solursch [218]. It may thus come as little surprise that the skeletal pattern, with a well-defined number of essentially similar elements in the proximo-distal direction, has also been interpreted in terms of the pattern-forming properties of reaction-diffusion systems.

'Stationary State Analysis' as an Approach to Reaction-Diffusion Modelling The major attempt at a reaction-diffusion approach to skeletal patterning, due to Newman and Frisch [269, 271], has employed a rather novel approach to reaction-diffusion modelling. They argue that model building in development must necessarily contain a strong hypothetical component. The standard approach, and the one we have seen applied in modelling throughout this chapter, is to postulate a specific, tractable, two-variable kinetic scheme, and to analyse its symmetry-breaking properties for comparison with the observed features that are to be explained. Such 'modelling' serves essentially illustrative purposes — it is intended to show that *some* reaction-diffusion system can generate the desired results. The alternative technique is to recognize that symmetry-breaking reaction-diffusion systems *are* physically admissible — such an approach

depends necessarily on the prior demonstration of the pattern-forming potential of reaction-diffusion systems indicated above — and, for a given situation, to seek to identify morphogens that might be governed by such reaction-diffusion schemes, the details of which are left largely unspecified. The challenge [271] is thus to seek what properties of the solutions of an incompletely specified reaction-diffusion system can be established, if for example only the existence of a stable patterned state, but not the rate functions, are known.

This approach to modelling is based on the contention that the behaviour of a reaction-diffusion system at the stationary state will often be independent of many details of the time-dependent process. Such models are thus formulated using assumptions about the qualitative nature of the dynamical processes involved, rather than a derivation of kinetics from first principles. Then biologically interesting features, such as steady-state concentration distributions allowed by tissue boundary conditions, can be studied in a system whose dynamics is incompletely specified. Such an approach may be favoured on the basis that, while the idea that morphogenetic processes might be governed by a reaction-diffusion mechanism is intrinsically reasonable, nonlinear reactions and spatial inhomogeneity being omnipresent in biology, knowledge of the detailed biochemistry and dynamical behaviour for any particular system is still profoundly incomplete.

The Newman-Frisch Model The analysis of a possible reaction-diffusion mechanism underlying chick limb development proceeded on the basis of such a 'stationary state analysis'. In the first paper on this work [269], Newman and Frisch assumed that the mesenchymal cells in the limb bud produce a morphogen, with concentration C, which at sufficiently high local concentrations could trigger cartilage condensation and differentiation in competent cells; its distribution pattern could thus provide a prepattern for the spatiotemporal development of the limb skeleton. On the basis of experimental evidence for its nonuniform distribution and involvement in limb patterning (and supported by subsequent work, see [271] and references therein) the adhesive glycoprotein, *fibronectin*, was identified as the possible morphogen, which at sufficiently high concentrations promotes the formation of limb primordia through local adhesive maxima. The distribution of fibronectin was assumed to be governed by a reaction-diffusion system, so that the possible spatial patterns were given by the time-independent equation for the stationary state:

$$D\nabla^2 C + R(C) = 0, \tag{4.33}$$

where $R(C)$ denotes the net rate of production of the morphogen. By means of a linear analysis, the possible solution modes were obtained, and compared to the successive condensation modes observed during limb outgrowth.

The initial work of Newman and Frisch [269, 270] was criticized by Othmer [297] on the basis that the model is too simple to generate the desired sequence of patterns; in particular, inhomogeneous patterns arising from single-morphogen reaction-diffusion dynamics are (nearly always) unstable (see appendix A.1.4, and the discussions in [271, 297]). In their later paper, Newman, Frisch and Percus [271] extended the model to include a further diffusible agent, which they tentatively identified as the secreted mesenchymal product, transforming growth factor β (TGF-β), to obtain a two-variable reaction-diffusion system (although now fibronectin was assumed to bind to the cell surface and not to diffuse). Again, however, a stationary state analysis was performed: With a general formulation of the kinetics, where only certain

constraints were given on the form of the incompletely specified rate functions, it was shown that the system behaviour depended, at the stationary state, on only one concentration variable. That is, for the postulated system

$$\frac{\partial C}{\partial t} = R(C,I) + D\nabla^2 C, \tag{4.34}$$

$$\frac{\partial I}{\partial t} = F(C - \gamma I), \tag{4.35}$$

where C and I are the concentrations of, say, TGF-β and fibronectin, respectively, at the steady state ($\partial/\partial t = 0$) the steady-state value of I can be found in terms of that of C from equation (4.35), and substituted into (4.34), so that the system reduces at the stationary state to a scheme of the form (4.33), that is, dependent on only one variable.

A linear analysis of (4.33) (which is generic for all reaction-diffusion systems) was performed in two (uncoupled) dimensions, with the distance from the previously specialized apical ectodermal ridge, or tip of the limb bud, being a parameter (compare the progress zone model [339], section 4.2.5; in the present model the existence of for instance a diffusible morphogen along the proximo-distal axis, which inhibits or triggers differentiation, is postulated). The problem was treated as a succession of independent stationary states, with no attempt being made to analyse the transient processes that connect one patterned state to another; this was not feasible, on account both of the difficulty of the mathematical problem of the transition between different inhomogeneous bifurcating solutions, and the incomplete specification of the reaction-diffusion system employed. The condensation patterns obtained from this analysis were found to agree qualitatively with the observed skeletal primordia, giving the correct number of concentration peaks.

Thus reaction-diffusion theory (albeit in a formulation that appears rather vague and ill-defined) appears to be able to account for chick limb chondrogenesis. Fairly little attention seems to have been paid to this approach, however, as an alternative and overtly more plausible scheme, based on the tractional interactions of mesenchymal cells, has been proposed [289] (see section 5.1.1). The major interest of the Newman-Frisch work seems to be not so much in the detailed application they propose, as in the novel modelling approach they employ.

Further Applications

The Work of Hunding An extensive series of analyses of the behaviour of the solutions of reaction-diffusion systems on a sphere and deformations thereof has been performed by Hunding, in collaboration initially with Billing. He has studied in particular a specific substrate inhibition scheme for glycolysis, proposed by Sel'kov (see [303, pp.164–166]). The determination of dissipative structures on a sphere has entailed both numerical [165] and bifurcation-analytic [33, 171] studies, and has required the development of enhanced numerical techniques (see appendix A.5) to handle the extensive computations required in three dimensions (see for example [170]). For completeness, and for extension to the biologically more realistic cases where perfect spheres are not observed, similar bifurcation studies were performed for prolate (elongated) [167] and oblate (flattened) [168] spheroids.

The biological application of these results is intended towards an understanding of the patterns underlying both cell division and development. The original application proposed a prepat-

tern theory of mitosis and cytokinesis (the division of the cytoplasm after mitosis): the orientation of the bipolarity, in pole and spindle formation, in mitosis and of the cleavage plane in cell division may be determined not by the centrioles (whose role as the primary spindle organizer has been questioned) but by a prepattern, established possibly by some reaction-diffusion mechanism. This suggestion was supported by the result that the first nonhomogeneous pattern established beyond bifurcation was shown, for the sphere as well as for spheroids, to be the bipolar pattern reminiscent of the orientation of mitotic division (for a discussion of the argument leading to the prepattern theory, with experimental support, see [166]).

Later work has postulated that the orientation of cleavage planes in early blastulas, which have a well-controlled spatial relationship to the animal-vegetal axis, may also result from the biasing of cytokinesis cleavage planes, through the predetermination of an axis by a prepattern [169]. In particular, coupled Turing systems can exhibit the controlled strict orthogonality required to generate the observed reproducible patterns of cleavage plane orientations; Hunding has found numerically that one reaction-diffusion system in three dimensions can stabilize another prepattern with its axis perpendicular to that of the former. The idea emerging from this work on reaction-diffusion systems on spheres is that the same mechanism has been exploited in nature to govern cell division and mitosis, as well as some processes in blastula and embryo organization, by simple modifications of some basic self-organizing mechanism (which need in fact not be a chemical prepattern — see the next chapter). The work of Hunding, in its particular focus on three-dimensional analyses, has stood essentially outside the mainstream of reaction-diffusion theory, although it appears to be a significant contribution to the understanding of the more difficult problems on the sphere and spheroids.

Catalano, Eilbeck and Coworkers A further predominantly numerical study (also involving the development of algorithms — see [91] and appendix A.5) is that of Catalano, Eilbeck and coworkers. The main emphasis of their work is towards the development of a reaction-diffusion formulation of an enzymatic system, and an analysis of its properties [53]. An allosteric model, in which a metabolite is produced at a constant rate and converted by an enzyme-catalyzed reaction into another species, is outlined, and assuming certain features and simplifications of the kinetics, these are formulated as a two-variable reaction-diffusion scheme. This is then analysed mathematically and simulated numerically, but no detailed attempt at correlations with specific biological systems is made. Rather, the symmetry-breaking properties of the system are pointed out, and compared heuristically with developmental situations. In particular, on the basis of two-dimensional simulations (instead of the complete three-dimensional analysis performed by Hunding) the onset of asymmetry in the egg after fertilization and resultant polarity of cell divisions in cleavage are related to the patterns observed in the solution of this reaction-diffusion system. As noted, this work is more of the nature of indicating a *possible* correspondence between symmetry-breaking in self-organizing systems and particular developmental situations, rather than an attempt to create a detailed link. As we have noted elsewhere (see appendix A.4) the work of Eilbeck, in particular, has provided important mathematical, rather than biological, contributions [44, 93].

Numerous other reaction-diffusion models with greater or lesser applicability to development undoubtedly exist. One example is provided by the study of a prepattern mechanism for spiral-type patterns of the sunflower head, by Berding *et al.* [32], which however falls outside our

chosen ambit of animal development. Another model, of dubious relevance, is that of Tapaswi and Saha [341], and in all likelihood several other applications have not been dealt with here. We have, however, attempted to cover all the major trends and applications, to provide a broad overview of the considerable usefulness and power of the chemical prepattern, self-organization paradigm in biological development.

4.3.5 Further Aspects of Reaction-Diffusion Mechanisms

As has surely become abundantly clear in the above discussions, in the context of the formation of chemical prepatterns reaction-diffusion models occupy pride of place; and rightly so, as the extensive analyses that have been performed have repeatedly confirmed the deservedness of their status as a paradigm for symmetry-breaking and self-organization. In the time-honoured tradition of science, the potentialities of this model have been fully explored, through the study of extensions and modifications, to determine the complete capabilities and ramifications of the concept of spontaneous chemical prepattern formation. Some of these studies and extensions will be discussed below, briefly.

Size-Dependence and Regulation in Reaction-Diffusion Models

Ever since Waddington's initial dismissal of Turing's theory [358], reaction-diffusion models for developmental patterning have been extensively criticized for their lack of size regulation [159], on account of the intrinsic chemical wavelength, which depends on kinetic and diffusion parameters but not on the size of the domain. The inherent scale in such prepattern models implies that the number of structures or pattern elements is size-dependent; doubling the size of a one-dimensional domain with multiple peaks of morphogen concentration, for example, will double the number of waves. This is in contrast to positional information, gradient models (see section 4.2), which were in general designed to ensure regulation and constancy of pattern.

Size-dependence may be a positive or negative attribute of a model, depending on the proposed application. There are numerous examples of structures whose numbers vary with size; these are generally structures which do not need to be accurately determined, including zebra stripes, hairs, *Drosophila* bristles, and hydra tentacles (for other examples see [159]). An important example is provided by the hairs in a whorl in *Acetabularia* (see [251]), where experimentation shows that the hair spacing is approximately constant irrespective of size, so that the number of hairs is proportional to the size of the plant. Such size-dependent patterning mechanisms are very suggestive of a Turing-type model or other, mathematically analogous, realization of local activation and lateral inhibition. Indeed, we have seen suuccessful examples of reaction-diffusion models for all of the above examples of size-dependent structures, and will in fact consider another model for *Acetabularia* hair patterns, also based on an intrinsic wavelength, though with very different underlying assumptions (see section 5.2.2).

Regulation There are, however, other important developmental situations in which the number of pattern elements is important, and can regulate with changes in size — the number of segments in *Drosophila* is an obvious example — for which the basic Turing-type reaction-diffusion mechanism appears to be inadequate. As has already been pointed out above (see

section 4.3.3), amendments to reaction-diffusion mechanisms or additional assumptions can be introduced that circumvent this problem. Thus, for instance, in a general kinetic system there are always reactants whose concentration is assumed constant for the reaction-diffusion kinetics — they are externally supplied and regulated — but which in fact influence the reaction rates and hence the chemical wavelength (see for example equation (3.34)). Allowance for a size variation of these concentrations, depending on their rates of delivery to and consumption within the morphogenetic region, lets one set up quite simple models in which the chemical wavelength can grow in proportion to the size of the system, so that one pattern can be stabilized over an indefinitely wide range of system size [150, 156]. Essentially, the crucial quantity for the scaling of any reaction-diffusion system is D/L^2, the diffusion constant divided by the square of the length of the system, which must be constant in order to have size invariance [13, 172]; here the diffusivity could however be an apparent diffusivity, which through the nondimensionalization includes reaction-kinetic constants.

A variety of models exhibiting size-invariance has now been proposed, most of which use some 'external' species influencing the rates, whose concentration is size-dependent. Thus Othmer and Pate [298, 302] introduce a regulatory species which affects the morphogen diffusivities, and whose loss through the boundaries of the region permits scale-invariance. Nagorcka [263, 264], as we have seen above, achieves invariance of *Drosophila* segment number in early nuclear division cycles by assuming a dependence of reaction rates and diffusivities on nuclear density n. Hunding and Sørensen [172] carry out a detailed study of size adaptation of Turing prepatterns, and show that the apparent diffusion constants in *normalized* reaction-diffusion systems may actually depend on rate constants which could be under control by external activators or inhibitors, through biochemical control systems, with concentrations depending on L^2; their estimates show that such control mechanisms, when appropriately combined with the reaction-diffusion kinetics, can give rise to size adaptation of cells over at least three orders of magnitude, which is more than sufficient to account for experimentally observed size variations.

Papageorgiou and Venieratos [301] chose a different route, achieving regulatory behaviour by imposing specific scale-invariance constraints on their reaction kinetics. Lastly, Babloyantz and Bellemans [11] argue that the lack of size invariance may be traced to the neglect of cell individuality in the continuum approximations. They consider purely topological factors such as cell packing and intercellular contacts, and show that (model-independent) size invariance arises naturally provided a change in size results in a reduction of the number of intercellular contacts or a decrease in the fraction of cell surface area in contact with neighbouring cells.

Thus mechanisms which deal adequately with size invariance have been proposed; any model can be made to display size adaptation if the parameters vary appropriately. Admittedly, the assumptions chosen to guarantee such scale invariance tend to appear somewhat *ad hoc*. Nevertheless, this is not a crippling defect in reaction-diffusion models, especially as the problem of regulation and size-invariance also plagues other (self-organizing) pattern-forming mechanisms.

Experimental Evidence for Morphogens

A fundamental problem that chemical prepattern mechanisms have had to contend with from the outset is the "reluctance of morphogens to stand up and be chemically identified" [150, p.381]; the lack of experimental confirmation and chemical characterization of pattern-determining

molecules has led to scepticism on the part of many experimentalists, and reluctance to consider reaction-diffusion and other models seriously. The true test of prepattern theories would be to isolate and characterize chemical substances that have demonstrable morphogenetic effects, and formulate molecular-kinetic models of their interactions to establish and test the correspondence between the model solution behaviour and observations. This is, of course, expected to be a challenging task, as such morphogenetic substances, if they are present, may be found in minute quantities [87].

Fortunately, as we have seen in the above discussions, the first signs of experimental confirmation are appearing. In particular, we have already noted, in our discussion of experimental observation of gradients (section 4.2.4), the evidence for head and foot inducing and inhibiting substances in hydra, as well as the evidence for retinoic acid in the chick limb. Extensive studies of the gene expression patterns and the corresponding protein products in *Drosophila* are also uncovering spatial inhomogeneities, which have stimulated a diversity of reaction-diffusion models, as we have seen. And calcium in particular has displayed significant pattern-forming potential, which has prompted the above reaction-diffusion model of *Acetabularia*, and which will be considered extensively in the discussion of mechanochemical models of cytoskeletal interactions, for instance in the cell cortex, that will be treated in the next chapter (see section 5.2). These are the most prominent examples; some other signalling or pattern-influencing substances are also coming to the fore (such as fibronectin and TGF-β appearing in a reaction-diffusion model for the chick limb). It appears that with the growing experimental support for chemical patterning, prepattern models will gain in legitimacy and popularity.

Discretization of Reaction-Diffusion Mechanisms

Discrete Cell-Cell Transport The analysis of discrete, rather than continuous, coupling between adjacent cells through diffusion or other chemical transport, or by other membrane-mediated interactions, has already been referred to in our discussion of positional information mechanisms (section 4.2.5). Babloyantz has shown [9] that for spatio-temporal organization or pattern formation to occur, it is not necessary that chemicals move through the field. Molecules in one cell can influence the rates of reaction in neighbouring cells by surface contact interactions without the actual passage of molecules from one cell to another, providing the necessary intercellular influences. She shows that the kinetic equations describing the time variations of the concentrations of chemicals within each cell (if reaction rates depend linearly on the concentrations of the relevant chemicals in the adjacent cells, but otherwise nonlinear interactions between molecular species within the cell are permitted) are formally identical to a system of reaction-diffusion equations in which the diffusion is approximated by discrete permeation of the chemicals from adjacent cells. Indeed, in the limit as the number of cells in the system tends to infinity and their size vanishes, the equations reduce simply to a set of partial differential reaction-diffusion equations.

All above analyses are thus valid, so that by means of such cell contact interactions, all self-organizing properties of reaction-diffusion systems may be obtained [9]. In the case where the rate dependences on the concentrations in adjacent cells, that is the *contact interactions*, are nonlinear, one might expect that even more complex behaviour may be obtained. Such contact interactions further embody the advantage that there is no need for long-range transmission of information, and transport of molecules; so that the size of morphogenetic molecules becomes

immaterial. Still, the long-range correlations that are characteristic of self-organization are observed, as the continuous time dependences and coupling between the reaction rates ensure that variations at one end of the system are rapidly (in the continuous limit, instantaneously) transmitted throughout the system.

Comparisons between Discrete and Continuous Formulations Othmer [296] has analysed the nature of the continuity assumption that is made in the mathematical treatment of reaction-diffusion systems. He notes that whereas discrete and continuous models may agree as the cell size tends to zero, and whereas a discrete formulation may be obtained simply by discretizing the Laplacian operator in the continuous description, nevertheless a continuum model must be recognized to be an *averaged* one on the macroscopic scale, which cannot be used for spatial variations on the scale of a cell. Ultimately, a discrete formulation which is able to take into account the underlying spatial structure and 'graininess' of the system, due to cellularity, must be more accurate than one which ignores all spatial inhomogeneities.

In his analysis, Othmer [296] points out that the two formulations can, in fact, produce different results: for example, if the kinetics have multiple steady states, the discrete description of two weakly-coupled cells can result in a stable non-uniform concentration distribution with only one chemical species, which is impossible in the continuum case (see appendix A.1.4). Furthermore, if a continuous reaction-diffusion equation is discretized to obtain the steady states, the resultant nonlinear algebraic equations may have solutions that vanish as the mesh size tend to zero, and thus do not approximate any corresponding solutions of the partial differential equations. Thus he emphasizes the need to consider the relationship between the two descriptions more carefully; to illustrate his point further, he displays some results on averaging the discrete case to obtain a continuous formulation that do not always correspond to the commonly or intuitively expected results.

In the light of this work, the salient point is important to note: While a continuous reaction-diffusion formulation may display wide and biologically suggestive solution behaviour, and is convenient in the light of the available analytical techniques, of bifurcation and stability analysis, such a formulation is not always the most appropriate in the light of the fundamental cellularization of the domain. Indeed, more diverse and complex solution behaviours may frequently be obtained in a discrete formulation. With respect to this point, recall also the numerical results of Harrison and Tan [156] on the switching behaviour of a reaction-diffusion system when a spatial scale for computations of the order of intercellular or internuclear distances was used. The effects of discretization of models will be considered in more depth in section 6.2, in the discussion of discrete systems and cellular automata.

Other Extensions of Reaction-Diffusion Mechanisms

The Role of Convection Various authors have discovered aspects of reaction-diffusion mechanisms that might be amenable to extension. Smith [329] has examined the possible role of convection on reaction-diffusion instabilities in a viscoelastic medium, by using the standard continuum theory of a fluid mixture. Through the use of linear stability analysis, he concludes that to first order in concentration changes, reaction-diffusion instabilities and patterns are unmodified by induced convection, and postulates that such a result would similarly be supported by a

nonlinear analysis. Numerical estimates and dimensional analyses support the conclusion that convection may essentially be ignored in reaction-diffusion mechanisms for pattern formation in embryology. This study thus vindicates the tendency in all the reaction-diffusion prepattern studies we have considered to neglect convection as an important transport mechanism. We should note, however, that in the consideration of mechanical instabilities and deformations, to be treated in the next chapter, convection may *not* necessarily be neglected.

Cross-Diffusion Effects In a recent extension to reaction-diffusion models, Almirantis and Papageorgiou [2] have challenged the assumption that diffusion must necessarily be scalar; that is, that the diffusive transport rate of a chemical species depends only on its own concentration. A (hypothetical) cell-contact membrane mechanism was studied, involving transmembrane microstructures endowed with two active and one regulatory sites, and the potential for enzymic action. The transport phenomena possible for such full cell-cell contact coupling were shown to include cross-diffusion effects (that is, non-zero off-diagonal terms in the diffusion matrix D) and the possibility of self-diffusion coefficients that are not necessarily positive.

The minimal requirements for pattern formation were examined for such a system, and it was shown [2] that bi- or even monomolecular reaction systems in a simple catalytic reaction are able to create patterns if cross-diffusion participates in the process, whereas, as we have seen above (see section 3.2.3) for pure self-diffusion, at least one trimolecular reaction is necessary for structure formation [357]. Bifurcation analyses and numerical simulations were performed, and hypothetical reaction schemes exhibiting the desired behaviour were proposed, both for the cases of weak (but non-zero) cross-diffusion, and for strong cross-diffusive coupling; and it was shown that cross-diffusion can generate stationary, but not time-dependent, dissipative structures and patterns. Further investigation of this situation is required, especially with a view towards experimental realization of cross-diffusion, to test in particular the important prediction that in the presence of cross-diffusion, "almost any two-variable reaction-diffusion systems can subsist destabilization towards patterned perturbations" [2, p.308]; as failing this, these analyses of cross-diffusive self-organization must remain novel theoretical curiosities.

Rules for the Interactions of Cells and Morphogens Finally, it should be noted that any number of models may be formulated by the introduction of rules for the mutual interactions of putative morphogenetic chemicals and cellular responses. For example, Khait and Segel [191] have proposed a model in which two chemical species, corresponding to a short range inducer and a long range inhibitor, are involved as signal carriers, but with no direct interaction between the morphogens. Rather, the continuing interplay between the cellular response to its chemical environment and the cell's secretion behaviour is emphasized, so that chemical and differentiated patterns are established simultaneously; cellular memory is also included. The model behaviour is formulated as a set of assumptions and rules which are iterated at successive time steps; in this particular formulation, linear model equations are obtained. Other assumptions can probably be readily introduced, and would yield different formulations and different pattern forming behaviours.

The value of such a hybrid model, being a mix of chemical prepatterns, cellular responses and developmental algorithms, is not quite clear, but it emphasizes some important points about chemical prepattern models in general: The constant interplay between cellular and chemical

responses in this model constitutes an internal regulation mechanism, including an implicit *feedback control* (each cell regulates the production of morphogens by its developmental level, which in turn depends on the environment that is created by the cells themselves). Hence here we no longer have the two-step process underlying positional information and prepattern mechanisms, in which cell differentiation (and other responses, such as deformation, movement or growth) follows as a slave process on the chemical concentration pattern, without influencing it at all.

Stability and Simultaneous Development The two-step positional information and prepattern mechanisms have been questioned or criticized precisely for their lack of feedback controls. A situation in which a prepattern exists and morphogenesis *then* takes place is essentially an 'open loop' system, with no regulatory interactions [287]. There would be no possibility of corrective feedback for the embryo to make the necessary adjustments to the inevitable disturbances during normal development, rendering such prepattern processes potentially unstable, and development unreliable. (Note however that the chemical patterning process itself is stable, through the mutual autocatalytic and feedback interactions between, say, activator and inhibitor.) Embryonic development is, however, usually a very reliable process, with embryos quite capable of buffering their development against many disturbances; this points towards an ongoing dialogue between changing patterns of chemical inhomogeneities, and morphogenetic movements and other responses [251, 287].

Such considerations strongly suggest *simultaneous development*, in which the cells themselves play a role in the patterning, with a continuous interaction between chemical and mechanical processes, differentiation, growth and other aspects of morphogenesis; such mechanisms have much more potential for self-correction. This does not by any means invalidate our analyses of positional information or prepattern mechanisms given above, however; they may still play fundamental individual roles in the sequence of processes that ultimately give rise to pattern and form in the embryo. The concern of modelling at this, still elementary, stage in our understanding of development is to explore the *entire* range of possible mechanisms and their potentialities, and we *have* noted numerous strengths (and weaknesses) of positional information and chemical prepattern models.

The above stability considerations have, however, now led us on to the models for simultaneous pattern formation and morphogenesis in the next chapter; these will again be considered within a general symmetry-breaking, self-organization paradigm, but from now the intimate interplay between patterning processes and cellular responses will be paramount.

Chapter 5

Cellular Activities in Self-Organization

Our previous studies have focussed on pattern formation and morphogenesis as two separate processes: first some positional information or prepattern, usually specified chemically, is laid down, and then the cellular responses are presumed to follow; cells at particular positions acquire particular properties. While a wide range of patterns may be accounted for by such mechanisms, we have noted some problems, both of experimental identification of the morphogens, and of stability, in that a slaved response of cells to a predetermined pattern embodies no potential for self-correction in the mechanisms, for adjustment to disturbances.

A fundamentally different approach to the analysis of structure formation blurs the distinction between pattern formation and morphogenesis; cells themselves participate in the pattern-forming process, so that pattern formation and morphogenesis occur *simultaneously*. The analysis of a wide range of cellular properties has shown that the mechanical activities of cells can themselves accomplish the morphogenetic functions usually attributed to chemical prepatterns, in particular the reactions and diffusion of (often hypothetical) chemical 'morphogens'. From the repertoire of embryonic cell activities, it is clear that movement, changes of shape and other mechanical interactions, often combined with chemical processes, are intimately involved in development, particularly in structure formation. We may thus generalize mechanisms of embryological pattern formation to include mechanical (vector and tensor) properties, instead of artificially restricting ourselves to the simple (scalar) property of chemical concentration. There is no *a priori* need to separate pattern formation and morphogenesis, and a more holistic, unified approach seems philosophically more satisfactory; as argued strongly by Harris [146].

A range of models analyzing and demonstrating the self-organizing behaviour of cellular activities has emerged over the last decade or so, in response to this paradigm shift. These have not invalidated the prepattern models of the last chapter, but caused a re-evaluation of their status from *the* mechanism for spontaneous pattern formation to *one* mechanism among others. We shall see that in many cases the predicted types of patterns are similar for different models, which is a reflection of frequently analogous mathematical formulations, and in particular an underlying conceptual similarity: local activation and lateral inhibition, in some form or other, play a basic role in many pattern-forming mechanisms (see [287]).

Morphogenetic Properties of Cells and Molecules We shall find that the study of cellular properties in development frequently requires us to pay closer attention to detailed biological properties of cells and molecules than we have had to previously. In the study of the morphogenetic properties of cells, it is convenient to recognize the distinction between the two main classes of embryonic cells, *mesenchymal cells* and *epithelial cells*. Mesenchymal (or fibroblast) cells tend to act *individually*; they are capable of independent movement, due to long finger-like protrusions called *filopodia* which grab onto adhesive sites on a tissue or external substratum and pull themselves along. Furthermore, they secrete the fibrous material that makes up the extracellular matrix along which they move, and interact with it, to generate forces and deform the matrix. Spatial patterns for mesenchyme appear as spatial variations in cell number density. We consider mesenchymal morphogenesis in section 5.1.

Epithelial cells, on the other hand, are prone to *collective behaviour*; they are packed together in sheets which can move, spread or thicken, or fold; they manifest spatial patterns through cell and cell sheet deformations. Owing to their fundamentally different properties, their pattern-forming mechanisms and resultant structures are quite different, and thus will be treated separately, in sections 5.2 and 5.3. Refer to section 2.1.3 for an overview of the properties of the different types of cells; Alberts *et al.* present an excellent general introduction [1], while Bard gives a clear and comprehensive discussion of the morphogenetic properties of mesenchymal and epithelial cells [16].

We shall further see that an appreciation of how cells cooperate to form tissues requires an understanding of how the *structural molecules* of the cells and their environment guide, constrain, facilitate and generate cell behaviour. The three morphogenetically significant classes of molecules, which may be distinguished 'geographically', are those associated with the *extracellular matrix* (ECM), those that are components of the *cell membrane* and those that comprise the *intracellular cytoskeleton*. These molecules have active, directive functions in morphogenesis, in generating space, directing cell movement, changing cell shape and mediating cell adhesion; and more passive or permissive roles in maintaining tissue stability and integrity. Again, some aspects of these properties are treated in section 2.1.2, and in detail in [1, 16].

5.1 Individual Cell Behaviour — Mesenchymal Cells

The morphogenetic behaviour of mesenchymal cells is based on an intimate feedback relation between the motile cells and their environment, in particular the extracellular matrix (ECM), through which the cells are capable of spontaneously generating regular geometric patterns of cells and matrix. The interactions which help orchestrate cellular movement include contact guidance by the ECM, motion along chemical or adhesive gradients, and contact inhibition. The most important aspect however appears to be the *traction forces*, by which the cells can deform their environment by rearranging extracellular materials, especially the fibrous protein collagen, and propel themselves. The interactions we consider should account, in particular, for the condensation of mesenchyme, which forms a common prelude to more complex organogenesis and thus constitutes the most important process in the creation of mesenchymal structures during embryogenesis.

5.1.1 Cell Traction in Mesenchymal Morphogenesis

There are four obvious ways in which loosely packed mesenchymal cells may increase their density [16]:

- They may proliferate locally — this is probably too slow to account for the formation of condensations;
- They may lose the ECM that keeps them apart;
- They may increase their adhesivity, so that the equilibrium minimization of free energy would cause them to aggregate following random intercellular contact;
- They may move towards some focus.

The second and third of these possibilities are plausible, but the spatial distribution of aggregations — for example, why there are one or a few condensation centres rather than many randomly distributed clumps — would need to be explained, possibly by means of a prepattern mechanism. Of the repertoire of available mechanisms, only **traction**, the tensile force that cells exert on their substratum and on other cells, has unequivocally been shown *in vitro* to generate condensations from uniformly distributed mesenchymal cells.

Experimental Effects of Traction The ability of cells to exert large traction forces able to wrinkle silicone substrata and create strong distortion patterns in collagen gels was first demonstrated by Harris, Stopak and Wild [148]. Later work by Stopak and Harris [335] demonstrated the morphogenetic significance of traction *in vitro*: Fibroblasts migrating out from tissue fragments were found to move towards one on the gel another owing to the alignment, due to tractional forces, of the collagen fibrils of which the gel was composed; cells moved preferentially along the 'tracks' thus created, due to contact guidance. They proposed that the tension, compression and alignment patterns observed *in vitro* revealed the mechanism by which tissues such as ligaments, tendons and muscles were aligned during development. For such results to be relevant to morphogenesis, however, we need to know whether the tractional effects demonstrated *in vitro* also play a role *in vivo*. The observation that collagen injected into chick limb buds was aligned by contractile forces within the developing tissues [336], supported by similar results, is compatible with such an assumption. Harris and his coworkers have argued strongly in favour of a significant morphogenetic role for traction (see for example [147]), and a theoretical explanation for traction-based condensation has been provided by Oster, Murray and coworkers, an explanation we shall now consider.

Mechanical Model for Mesenchymal Condensation

The basic mechanical model for mesenchymal condensation is based mainly on the two properties of mesenchymal cells *in vivo* discussed above:

1. Cells migrate within a substratum consisting of fibrous ECM and other cells, and
2. Motile cells can generate large traction forces on the ECM.

These and other factors that influence mesenchymal cells to migrate are encapsulated in the model, which is formulated (in continuum form, that is, not taking account of the discrete nature of the cells) in terms of three differential equations, governing the conservation equation for the cell density, the mechanical balance of forces between the cells and the ECM, and the conservation law for the ECM.

Factors Included in the Model Numerous factors affecting cell motion may be taken into account in the model. Those that have been incorporated in the formulation we consider include:

- Random, undirected motion down a cell density gradient, that is, diffusion (both short and long range);
- Convection, whereby cells may be carried passively along a deforming ECM substratum;
- Haptotaxis, where cells move up an adhesive gradient due to a gradient in ECM density, and thus in the density of adhesive sites; and
- Contact inhibition, where a high density of neighbouring cells reduces tractional effects and thereby inhibits motion.

Other factors that could be taken into account, but have been neglected here, include contact guidance, where the motion of cells follows geometrical cues in their substratum, in particular the alignment of the matrix; chemotaxis, or directed cell motion in response to a gradient of some chemical; and galvanotaxis, where the electric potentials known to exist in the embryo provide a preferred direction of motion (see for example [255]). It is clear that a multitude of factors could be incorporated into the model, rendering the analysis quite intractable, and making it difficult to differentiate between the morphogenetic effects of different terms. Thus it is in fact preferable to treat different factors separately, in order to gain qualitative understanding of the results.

Cell proliferation, or mitosis, can also be incorporated into the cell conservation equation. For matrix conservation, on the other hand, only convection, and possible secretion of matrix by cells, is taken into account. Finally, the force balance equation treats the matrix as a viscoelastic material: owing to the long time and small distance scales during development, inertial effects in the mechanical equation may be ignored, and the traction forces generated by the cells are taken to be in mechanical equilibrium with the viscoelastic restoring forces and any external forces present.

The consideration of the above factors leads to the mechanical model for mesenchymal morphogenesis proposed by Oster, Murray and Harris [259, 289]. Many reviews of this model by the above authors now exist; for a detailed introduction, see for example [251, 255], with more heuristic, biologically oriented treatments given in [254, 287, 288]. A derivation and discussion of the various terms of the equations, with some aspects of their solution behaviour, is given in appendix C. For illustration only, typical model equations for the cell density n, matrix density ρ and matrix displacement $\mathbf{u}$, are given below:

Cell conservation:

$$\frac{\partial n}{\partial t} = -\nabla \cdot \left[n \frac{\partial \mathbf{u}}{\partial t} \right] + \nabla \cdot [D_1 \nabla n - D_2 \nabla (\nabla^2 n)] - \nabla \cdot [a_1 \nabla \rho - a_2 \nabla^3 \rho] + rn(N - n); \tag{5.1}$$

Cell-matrix mechanical interaction equation:

$$\nabla \cdot [\mu_1 \varepsilon_t + \mu_2 \theta_t \mathbf{I} + E'(\varepsilon + \nu' \theta \mathbf{I}) + \tau n (1 + \lambda n^2)^{-1} (\rho + \gamma \nabla^2 \rho) \mathbf{I}] - s \rho \mathbf{u} = 0; \tag{5.2}$$

Matrix conservation equation:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}_t) = S(n, \rho, \mathbf{u}). \tag{5.3}$$

(For the meanings and derivations of the various terms and parameters, see appendix C.)

Basic Solution Properties of these Equations As is evident from the above equations, and as indicated in the appendix, the behaviour of the equations is extremely complex, and the analysis has hardly begun to explore the full range of features demonstrated by this highly nonlinear set of equations. The salient feature of present interest is that a linear stability analysis of these equations for small perturbations about the (nontrivial) homogeneous steady state n and ρ constant ($n = \rho = 1$ after nondimensionalization), $\mathbf{u} = 0$, yields a series of dispersion relations giving the ranges within which the steady state is unstable, and spatially nonuniform structures can form. This analysis is essentially like that for reaction-diffusion equations, and analogous symmetry-breaking bifurcations may occur, giving rise to spontaneous patterning, that is, self-organization.

It should be noted that these equations in fact exhibit a far greater range of solutions than is possible for reaction-diffusion equations, as is apparent from the diversity of dispersion relations (see [257]), so that reaction-diffusion-generated patterns form merely a subset of those theoretically possible through these mechanical interactions. Nevertheless, the same basic principles of self-organization apply. The interpretation of the mechanical interactions in terms of local autocatalysis and long-range inhibition (see appendix C.2), which places the mechanical models squarely within the class of LALI models [287], provides a basis for this qualitative equivalence. A clear biological explanation for the pattern formation mechanism is found in [147].

Particularly noteworthy is the fact that, in the formulation of the mechanical model — see appendix C, and for more details refer to the original papers and the book by Murray [251] — cell traction plays an indispensable role in pattern formation, being a participant in all destabilizing terms. This accords well with the biological observations described above [147, 148], and motivates the use of traction as the natural bifurcation parameter in the model, compatible with the observation that traction can increase during the lifetime of a cell. Such qualitative considerations, combined with the analytical power of a detailed mathematically formulated model for mesenchymal morphogenesis, have led to a number of particular applications for the involvement of fibroblast cells in the development of vertebrate pattern and form.

Integumental Patterns

Motivation — the Comparison with Reaction-Diffusion Models We have seen a range of reaction-diffusion-based models for integumental patterns, including animal coat patterns [14, 247, 248], and skin organ primordia [262, 266, 267], which were able to reproduce major characteristic observed features. A notable property of these models was the significant dependence on geometry and scale of the domain at the time the prepattern was laid down (for a discussion, see [251]), from which certain interesting predictions and developmental constraints could be deduced. The mechanochemical models discussed above deal with pattern formation through migratory cells, — these could correspond for example to melanocytes arising from the neural crest — and so could be directly applicable to coat patterns.

The solutions of the partial differential equations in terms of which the mechanical models are formulated (see appendix C) display similar domain and boundary dependencies to reaction-diffusion equations, and are thus expected to lead to analogous developmental constraints. Indeed, analysis of the wide range of dispersion relations possible for the mechanochemical equations reveals that a great diversity of patterns may be created, of which those possible by a reaction-diffusion mechanism are only a subset. Thus integumental patterns form a natural field of application for the mechanochemical models.

In our studies of reaction-diffusion equations, we noted that the regularity of the pattern spacing frequently depended critically on the mode of pattern initiation; simultaneous patterning tended to result in less regular spacing between morphogen concentration peaks than sequential laying down of the pattern. The partial differential equations of the mechanochemical models display similar properties. Thus one might expect, using for example traction as a bifurcation parameter, that the fundamentally indeterminate mammalian coat patterns and hair fibre spacings would correspond to a situation in which the traction exceeded a critical value simultaneously throughout the domain of interest, so that the 'stochastic indeterminacy' resulting from the dependence on the initial perturbations leads to the slight variations that render every animal unique. The traction-based explanation for such irregular patterns is supported by the experiments of Harris *et al.* [147], who were able to generate slightly irregular, periodic, spontaneous condensations of mesenchymal cells *in vitro*, through a mechanical instability such as that discussed.

Periodic Patterns of Feather Germs In contrast to the irregular patterns of hair and coat markings, feather and scale germs display a striking regularity, being distributed across the surface of the animal in a characteristic hexagonal fashion [73]. An early application of the mechanical models described above was to feather germ primordia [259, 289]. The model attempts to account for the formation of dermal papillae, that is, the condensations of mesenchymal cells in the skin dermis that precede feather formation. The skin essentially consists of motile *mesenchymal dermal* cells beneath an *epithelial epidermis*, which can deform through buckling. Each feather primordium consists of a thickening of the epidermis, called a placode, beneath which is a papilla, an aggregation of dermal cells. Considerable experimental work involving dermal-epidermal recombination experiments has been performed to determine whether the dermis or epidermis controls the spatial patterning; a model invoking mesenchymal morphogenesis is based on the assumption that the dermis plays the crucial role.

As indicated in appendix C, appropriate solutions with hexagonal symmetry have been established for particular cases of the mechanical models [216]; these could correspond to the desired patterns, but are likely to be very special cases requiring particular symmetries in the initiating perturbations, and thus to be highly unlikely relative to more irregular patterns, such as obtained experimentally by Harris *et al.* [147]. As noted in that work, however, *sequential* patterning would lead to more regular patterns, and indeed it has been demonstrated that chick feather primordia appear sequentially, with a 'wave of determination' establishing the positions of primordia immediately preceding the visible formation of epidermal placodes (which are observed before the dermal condensations) [73].

The model explanation based on these observations is a quasi-one-dimensional one: Each row of papillae is formed individually, with the spacing of condensations determined by the solutions to the mechanical equations in one dimension — numerical simulations [306] confirm that the full mechanical model predicts regular one-dimensional patterns. As the papillae form, tension lines develop [335], joining the cell aggregation centres, consistent with cells trying to align the ECM. The matrix strains thus set up by the initial row of papillae then bias the formation of the secondary condensations, so that the lateral rows of papillae forming adjacent to the initial row are interdigitated with the papillae in the preceding row; successive secondary condensations are displaced from the previous ones by half a wavelength, again in agreement with numerical simulations [306]. This scenario thus generates a regular hexagonal pattern in a sequential way like a wave emanating from the dorsal midline. The 'wave' is more like a kinematic wave, however, since if the dermal layer is cut along a line parallel to the dorsal midline the wave starts up again beyond the cut *ab initio* [74].

Thus a plausible foundation, based on quasi-one-dimensional simulations and some experimental results, has been proposed for the generation of feather primordia as an example of morphogenesis by traction-based mesenchymal condensations. It should be noted, however, that this mechanism has been questioned, on the basis that dermal-epidermal recombination experiments suggest strongly that the *epidermis* plays a central role in determining where the dermal condensations will be (for a review, see [16]). The evidence points to the view that inductional interactions between dermis and epidermis generate a prepattern of cell adhesion molecules in the epidermis (CAMs are already present *during* condensations, whereas mesenchymal condensation prior to biochemical changes can only explain N-CAM expression *after* papillae have formed). Thus there in fact seems little need to invoke a traction-based mechanism for the primary morphogenesis of dermal condensations, although traction could of course play a secondary role in ensuring the compaction of the condensations, and mediating the alignment between formed condensations that occurs after aggregation [17].

Complex Integumental Patterns In both the reaction-diffusion and mechanochemical models of integumental pattern formation considered so far, there has been no strong theoretical support for the formation of complex patterns, which occur for example in the scales of some reptiles, where a regular scale pattern appears to be made up of a superposition of two patterns with different basic wavelengths. From the dispersion relations studied, there is usually a dominant wavelength, or a range of unstable wavelengths which would somehow be superimposed on the final pattern, but little evidence for two distinct unstable patterns whose wavelengths differ by a factor of at least two. Such complex patterns may be treated and accounted for theoretically through the coupling of two pattern-forming mechanisms with independent dispersion

relations, each with a set of unstable wavenumbers but of different ranges.

Nagorcka *et al.* [265] have investigated complex scale patterns by considering an integrated mechanism consisting of the interaction between a reaction-diffusion model for the epidermis with a dermal cell traction model for the dermis; mathematical analyses of the model have been performed by Shaw and Murray [320], and by Maini [215]. The results suggest that if the coupling is weak, the composite mechanism will be unstable to perturbations at scales with wavelengths approximately equal to the unstable wavelengths which characterize the two mechanisms independently, provided these are sufficiently well separated. Thus such interactive mechanisms and coupling (which could of course, for example, also be between two two-component reaction-diffusion systems) provides a basis for understanding the generation of yet more complex patterns from those possible through each individual mechanism.

Cartilage Condensations in Limb Morphogenesis

We have already noted that the vertebrate limb is one of the most easily and widely studied developmental systems (see section 2.2.2), and models based both on positional information (with the debatable role of retinoic acid) and reaction-diffusion were discussed in the previous chapter. Another significant application of the mechanical theories of mesenchymal morphogenesis has been to chondrogenesis, the generation of the pattern of cell condensations which eventually become cartilage. Such models are based on the fact that the cellular patterns in developing limb buds which determine the final cartilage patterns, which later ossify into bones, involve aggregations of mesenchymal cells. Thus a mechanical model such as the one we have been considering [259, 289] could generate condensations of mesenchyme corresponding to the bone rudiments.

Mechanochemical Model Oster, Murray and Maini [290] proposed a similar model in which no active cell motility was considered, only passive convectional dragging along the deforming ECM. Instead, other mechanochemical interactions known to occur *in vivo* are incorporated into the model: Hyaluronic acid (HA), a principal component of the ECM, can exist in a swollen osmotic state, which swells the limb bud and keeps the cells apart. At a certain stage in the development of chondroblast cells, possibly as they have emerged from the progress zone and aged sufficiently, or as the concentration of HA has reached a critical level, the cells begin secreting an enzyme, hyaluronidase (HAase), which breaks down the HA, causing the interior of the limb bud to deswell. This could lead to osmotic collapse of the matrix, bringing the cells into close enough contact to exert strong traction forces. These could deform the ECM and sufficiently drag other cells in to initiate active contractions through the interplay between osmotic deswelling, tissue elasticity and cell tractions; these contractions can generate organizing centres leading to the condensation of cells into the dense aggregations which form limb rudiments as the beginnings of chondrogenesis.

The mathematical formulation of such a model incorporating these mechanical and chemical interactions is considered in appendix C.2; where it is also shown that, under certain simplifying conditions, the model equations can reduce to a form analogous to reaction-diffusion equations, and thus that the model displays at least the self-organizing, patterning behaviour we have learnt to associate with reaction-diffusion systems.

The Generation of a Sequence of Condensations There is no direct way at present of deciding between the above two mechanically-based scenarios for limb patterning, or indeed between these and the reaction-diffusion models. In contrast to reaction-diffusion models, however, the mechanical models suggest an interesting and plausible scheme for the generation of the appropriate sequence of condensations, based on the critical dependence of pattern on geometry and scale: As the limb bud grows, through cell proliferation at the distal end, the cross section of the tissue domain (which includes the ECM and mesenchymal cells) is considered to be the two-dimensional domain for the mechanical model; it is approximately circular, but with an elliptical bias. We suppose that as the cells age the traction increases, until it passes through the critical value τ_c and the first bifurcation occurs; the domain size is assumed to be such that a single central aggregation of cells recruited from the surrounding tissue is formed. As the cells condense, they generate a strong, radially-directed stress, which *deforms* the already slightly elliptical cross section to make it even more elliptical. Since the aggregations are influenced by the cross-sectional shape, the changed flatter geometry induces a secondary bifurcation to two condensations, which may have different sizes if the limb cross section is not symmetrical, but more aerofoil-shaped. Further growth and flattening can generate more distal patterns through successive deformations and bifurcations.

The salient feature here is that the mechanical models incorporating traction and stress, as distinct from chemical prepattern models, directly influence the shape of the domain and can thus actually *induce* the required sequence of bifurcations. Variations in model parameters other than the geometry could of course also generate the sequence of bifurcations; but the changing domain geometry, accounted for within the model, is an aesthetically pleasing method of generating the desired patterns. Between one and two condensations, there may also be a region in which no pattern elements fit in, and the uniform solution is stable (we observe similar behaviour for reaction-diffusion models, where a domain size above the critical size for patterning could be such that no allowed wave numbers are unstable — see for example [199]); this could correspond to the region of presumptive joints between different cartilage elements.

Developmental Constraints in Chondrogenesis Whatever the trigger of the sequence of bifurcations as we move from the proximal to distal part of the limb, and indeed whatever the detailed nature of the self-organizing model used to describe the observed sequence, the natural progression is from one to two to several condensations. For absolute tissue isotropy and a completely symmetric domain geometry, it is conceivable that one moves through the bifurcation space of parameters from one aggregation to two, to three, and so on, but such perfect symmetry is highly unlikely in nature. Hence for all practical purposes a triple bifurcation must arise from a process of branching bifurcation from one branch of a double condensation. Such considerations of the feasible bifurcations in a real developing limb have led to the conclusion that all limb patterns arise from one of three basic types of cell condensations, namely a localized, *focal condensation* (or initial aggregation), a *branching bifurcation* and a *segmentation condensation* (in which growth of the domain causes a lengthy aggregate to break up).

A set of *morphogenetic rules* for the patterning sequence of cartilage in the developing limb has consequently been hypothesized, which constrains the limb cartilage patterns which may be formed, thus providing a developmental constraint analogous to the one restricting possible coat patterns [248] (see section 4.3.2). These rules are essentially model-independent, but in the sense that any model mechanism for chondrogenic condensations, such as those we have

considered, should be capable of generating such a sequence of bifurcating patterns. Here very general considerations of models based on lateral inhibition, or self-organizing mechanisms, thus suggest constraints on the evolution of the vertebrate limb, in that the possible shapes and cartilage geometries that could evolve are restricted by the developmental mechanisms used to create the limb. Extensive studies have revealed considerable experimental evidence for these rules (for a discussion see [251]).

Hence a mechanical or mechanochemical cell traction-based model appears plausible as a basis for limb chondrogenesis, although a clear basis for discriminating between these scenarios and those based on chemical patterning [271] (see section 4.3.4) does not yet exist. The experimental evidence indicates that condensations can form *in vitro*, but is insufficient to prove that tractional aggregations have a role to play in the segregation and condensation of mesenchyme *in vivo* [16, 17], although on the other hand there is no evidence to contradict such a hypothesis. "In general it has to be admitted that our knowledge of bone morphogenesis is woefully inadequate." [16, p.161].

Somitogenesis

In the above discussion we have considered the two original applications of the mechanochemical paradigm, to the morphogenetic situations of feather germ formation and chondrogenesis [289]. In his extensive and experimentally-orientated reviews [16, 17] Bard has questioned these applications, as we have noted, regarding the status of knowledge on bone formation to be inadequate, and finding the evidence to contradict a dermal model of mesenchymal aggregation for feathers. Other plausible applications have, however, also been proposed, in particular pertaining to the segmentation of vertebrate somites.

We have already considered the 'clock and wavefront' prepattern model of somitogenesis [64] (see section 4.2.5), which assumes that sequential groups of cells, somehow temporally coordinated, acquire cues which cause them to cohere and, through mechanisms unknown, detach from the surrounding unsegmented mesenchyme. The molecular prepatterns involved might be expected to be, for example, the coherent expression of cell adhesion molecules (CAMs) in groups of cells. Recent studies have however shown (see [16]) that the adhesion molecules N-CAM and N-cadherin are continuously distributed and expressed in newly formed chick somites, well before segmentation takes place, with no evidence of differential expression to suggest that future somite borders will reflect regions where adhesion molecules are absent. Such observations pose a problem for prepattern models, but suggest an alternative function for the adhesion molecules: As they are expressed (which is in a sequential fashion, with a CAM gradient moving caudally down the embryo) the cells become highly adhesive and are in sufficiently close contact to exert strong tractional forces on one another. This tractioning could thus cause mesoderm to segregate in groups and form somites, in a manner analogous to the mechanism for chondrogenesis described above.

Traction-based Mechanism for Somitogenesis On this basis, Bard [15] has proposed a feasible model for chick somitogenesis as the traction-based segmentation of a mass of highly adhesive cells. This model is able to account for a number of otherwise strange experimental features of somitogenesis, such as the formation of multiple rows of somites in wide mesenchyme

(for a discussion, see [16, 17]). A mechanical model for somite formation has been questioned in favour of a prepattern model, in view of the observation that the visible segmentation process can 'jump across' surgical gaps in the tissue, created prior to observed aggregation, suggesting that some cryptic invisible pattern has already been laid down; for a mechanical model one would expect the proposed cell movements or condensations which form the patterning process to be visible under a light microscope [327]. Provided the future gradient of CAM expression is laid down before the cut is made, however, this is not a problem, as the tractional mechanism need then only be operative once the cells become sufficiently adhesive, with a kinematic wave of CAM expression inducing the wave of somite segmentation through tractional condensations. Somitogenesis thus provides yet another domain within which the mechanochemical scenario of simultaneous traction-based patterning and morphogenesis may become operative.

Other Possible Roles for Traction

The Formation of Nephrons in Kidneys Bard [16, 17] has considered another example of mesenchyme condensation, the aggregation of metanephric mesenchyme to form nephrons (the excretory tubules of vertebrates) in the developing kidney. This is a particularly interesting developmental system, as it demonstrates that mesenchyme and epithelium are not immutable: metanephric cells, that is the *mesenchyme* that will form nephrons, aggregate into condensations, at which point a complex sequence of molecular and cellular activities causes the cells to reorganize, to form the *epithelial* tubule constituting the nephron.

Preliminary experimental work has shown that if kidney rudiments *in vitro* are subjected to conditions, such as the presence of cytochalasin, that should reduce mechanical interactions but have no direct effect on gene expression, then condensation appears considerably inhibited. On the other hand, the known distributions of molecules from the ECM, the cell membranes and the cytoskeleton throughout nephric development are taken as evidence that prepatterns are unlikely [17]. Although the mechanism for segregation of metanephric mesenchyme is unknown, the plausible suggestion is thus that here, again, traction may well mediate the early stages of tissue and organ formation, in this case of nephrogenesis.

Wound Healing A final situation in which the participation of traction has been considered is not directly connected to primary embryonic patterning, but rather to the later response of the organism to injury; it is worth our brief consideration, however, as the mechanisms involved may be similar. In the process of cutaneous wound healing, fibroblast cells recolonize the wound area through migration and mitotic proliferation, generating large tractional forces which cause contraction of the wound boundary. The mechanochemical approach has been used as a framework for understanding the mechanism of wound healing, with the hope of being able to provide a predictive means for enhancing or mitigating contraction for a particular wound, with a view to minimizing scarring; preliminary work is reported in [255]. There are considerable potential benefits to gaining a thorough understanding of wound healing; thus a detailed study of the mechanochemical models we have discussed, with particular reference to wound healing, is highly justified [251]. It should be noted that an alternative model based on the biochemical regulation of mitosis, with mitotic proliferation and diffusion of fibroblast cells combined with the production, decay and diffusion of a mitosis-regulating chemical, has also been proposed and studied for epidermal wound healing [322, 323]. Once again, the challenge is for the detailed

analysis of the models and discrimination between their respective predictions, combined with careful, focussed experimentation.

Conclusion: A New Paradigm for Developmental Self-Organization The discussion of traction-based models for mesenchymal morphogenesis has been fairly extensive. This is may be traced partly to the diversity of proposed applications, but is especially due to this new approach of cellular participation in patterning, compared to the two-stage chemical prepattern and positional information models that we previously studied. It is particularly significant that interactions other than reaction-diffusion may display self-organizing properties and be interpreted in terms of lateral inhibition (see appendix C.2), and that in this case we have the advantage of *simultaneous* pattern formation and morphogenesis, with its expected concomitant enhanced robustness and stability. In contrast to chemical models, where the chemicals involved are frequently unknown, and the detailed kinetic mechanisms have nowhere been characterized, the parameters in the mechanical models are all, at least in principle, experimentally measurable, so that these models also have a potentially higher degree of experimental falsifiability, an important criterion for a theory in the Popperian sense.

The extension of self-organization models beyond reaction-diffusion systems provided a conceptual breakthrough, which opened the way for a proliferation of other models in which cells, rather than responding blindly to a predetermined chemical pattern, participate intimately in structure formation; in which pattern formation and morphogenesis go hand in hand. We shall proceed to investigate a range of such models, beginning with other models for mesenchymal behaviour in which more limited interactions of individual cells have been demonstrated to display pattern-forming, symmetry-breaking properties.

5.1.2 Chemotaxis Models for Pattern Formation

The formulation of the general traction-based model for mesenchymal morphogenesis developed and discussed in the previous section may potentially include a complete range of cell-cell and cell-matrix interactions, including cell-cell contact and inhibition, chemotaxis, haptotaxis, galvanotaxis and any other factors which may be deemed important for the application under consideration. Some of these interactions between cells only, in the absence of or neglecting the matrix, have also been shown to generate patterns under appropriate conditions. The fundamental idea underlying essentially all these models may be couched intuitively in terms of local activation and long-range inhibition of some form (see [287], and appendix C.2), revealing a deep equivalence among such self-organizing mechanisms.

Some form of directed migration, or taxis, is frequently involved in cell behaviour, as demonstrated in the haptotaxis (motion along an adhesive gradient) incorporated into our previous considerations. We consider now a model for *cell chemotaxis*, or motion in response to a gradient of some attracting (or repelling) chemical. This phenomenon has long been appreciated, especially in bacterial behaviour; a theoretical analysis for the response of motile *Escherichia coli* moving preferentially towards higher concentrations of oxygen, minerals and organic nutrients in search of an optimal environment, was already given by Keller and Segel in 1971 [186]. The partial differential formulation of this model was able to account for the observed travelling bands and waves, and generated further experimental and theoretical interest (see for example

[184, 261]).

The Keller-Segel Model for Slime Mould Chemotaxis

Probably the theoretically best-understood developmental system displays chemotactic behaviour; this is the cellular slime mould, *Dictyostelium discoideum*, already discussed in some depth in section 3.3. Much is now known about the mechanisms of cell-cell communication via cyclic-AMP (cAMP), and both the signalling process [356] and the slug motion [282] have been modelled in detail, incorporating biochemical reactions and diffusion. The combination of current theoretical insights has led to a fairly comprehensive, complex model from which the details that capture the essential features now need to be identified and emphasized [66].

Of interest to us here is the original, seminal model for the aggregation behaviour, which is now largely superseded in the light of later experimental studies, but which showed the way for the modelling of slime mould behaviour and indeed, realistic self-organization modelling in developmental biology in general. This is the model of Keller and Segel [185] (for a good pedagogic discussion, see [87]), which was based on a number of simplifying assumptions:

- Individual cells move by a combination of random motion and chemotaxis towards the chemoattractant, cAMP.
- Cells neither die nor divide during aggregation.
- The attractant cAMP is produced at a constant rate by each cell.
- The rate of degradation of cAMP depends linearly on its concentration.
- cAMP diffuses passively over the aggregation field.

Of these assumptions, only the third and fourth are drastic simplifications [87].

Model Formulation The above assumptions may be translated into a system of two partial differential equations depending on five parameters, in an analogous manner to the reaction-diffusion models derived earlier on in this thesis. The model system is derived and analyzed in detail in [87, 185], and will be treated only briefly here: We define the system variables $a(\mathbf{x},t)$, the density of cellular slime mould amoebae, and $c(\mathbf{x},t)$, the concentration of chemoattractant, in this case cyclic-AMP. For fluxes J, and restricting ourselves for simplicity to one spatial dimension, we obtain

$$\begin{aligned}\frac{\partial a}{\partial t} &= -\frac{\partial}{\partial x}(J_{\text{random}} + J_{\text{chemotactic}}) \\ &= -\frac{\partial}{\partial x}\left(-\mu\frac{\partial a}{\partial x} + \chi a\frac{\partial c}{\partial x}\right), \qquad (5.4)\\ \frac{\partial c}{\partial t} &= -\frac{\partial}{\partial x}(J_{\text{diffusion}}) + \text{ sources } - \text{ sinks} \\ &= -\frac{\partial}{\partial x}\left(-D\frac{\partial c}{\partial x}\right) + fa - kc, \qquad (5.5)\end{aligned}$$

where μ is the amoeboid motility, χ is the chemotactic coefficient, D is the diffusion rate of cAMP, f is the rate of cAMP secretion per unit amoebae density, and k is the rate of degradation of cAMP in the environment. In principle the quantities μ, χ, k and f may depend on cellular densities and chemical concentrations, but for an initial exploratory model it is sufficient to assume them to be constant parameters of the system. The terms in the above equations are all familiar from our previous work, except possibly the chemotactic flux; we note that its positive sign indicates amoeboid diffusion *up* a gradient in cAMP concentration.

Analysis of the Keller-Segel Model Analysis of this model proceeds in the manner to which we are by now accustomed. The homogeneous steady state, which gives the average or bulk cell and chemoattractant densities, is given by the constant solutions $a(x,t) = \bar{a}$, $c(x,t) = \bar{c}$, under the condition $f\bar{a} = k\bar{c}$ — that is, the rates of secretion and degradation are everywhere equal. Considering small perturbations from this steady state, we perform a linear stability analysis (see appendix A.2, or [87]). This analysis readily reveals that instability for a mode with wavenumber $q = n\pi/L$ (for a domain of length L) is possible provided

$$\mu(Dq^2 + k) < \chi\bar{a}f. \tag{5.6}$$

Interpretation of this aggregation condition indicates that spatial instability and aggregation are promoted by low random motility of the cells and a low rate of cAMP degradation; and correspondingly, by large chemotactic sensitivity, a high secretion rate of cAMP, and a high bulk density of amoebae, $\bar{a}$ [87]. These conditions agree well with observed influences on *Dictyostelium* aggregation, so that this simple model clearly reveals the possibility for instability and self-organization qualitatively consistent with the observed aggregation.

The analysis so far has only indicated that spatial instability driven by a chemotactic mechanism is *possible*, and is presented here for comparison with the more general chemotaxis models proposed recently for patterning, mainly on the integument of vertebrates. Further more detailed modelling, based on the cell-cAMP generation and signalling system, has been performed to give a detailed fit with experimental observations, including the prediction of circular and spiral wave patterns [356]. Much analysis of the other stages of the life cycle of the cellular slime moulds has been performed, making it probably the best-understood developmental system — see section 3.3 and references therein. The slime mould may thus be seen as a biological paradigmatic system for self-organization, and in particular chemotaxis, filling the same role as the Belousov-Zhabotinskii autocatalytic chemical reaction (appendix B).

Chemotaxis in Vertebrate Patterning

We are presently interested chiefly in the development of multicellular organisms, whose behaviour is clearly significantly more complex than that displayed by the (usually!) single-celled creatures described above. It is nonetheless possibly surprising, however, in the light of the above theoretical predecessors, that a chemotaxis-based model for vertebrate patterning was only first proposed by Oster and Murray in 1989 [288] (for a discussion, see also [252]), the formulation of which is described in appendix C.3. The model involves motile cells, of density $n(\mathbf{x},t)$, which secrete a chemical attractant, with concentration $c(\mathbf{x},t)$, to which the cells respond chemotactically by directional movement up a chemoattractant concentration gradient.

As usual, the model formulation, obtained in the appendix with a description of the parameters, involves two partial differential equations describing the conservation of n and c; an example of such a system, neglecting mitosis, is

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \alpha \nabla \cdot (n \nabla c), \tag{5.7}$$

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c + \frac{Sn}{\beta + n} - \gamma c. \tag{5.8}$$

The model formulation is completed by appropriate boundary conditions, for example zero flux conditions reflecting the fact that the cells and chemoattractant are contained within a finite domain, and initial conditions such as random perturbations from a homogeneous steady state. A heuristic explanation for the formation of heterogeneous patterns of localized clumps of cells is given in appendix C.3, together with an interpretation of the chemotactic mechanism in terms of the lateral inhibition paradigm.

Integumental Patterns — Again! This mechanism has been applied to a patterning situation we have already frequently encountered, and will consider later in yet other contexts — integumental markings. In this case, the motivation for the application is based on the behaviour of neural crest cells, the precursors of pigment-forming melanocytes, which migrate over the surface of the embryo, and are considered to respond chemotactically to a chemical gradient. Stripes or spots of pigmentation are assumed to correspond to local aggregations of melanocytes, in patterns predicted by the model equation solutions.

Murray and coworkers have considered an application to alligator stripe patterning, in conjunction with extensive experimentation [253]; the basic idea is that the stripe pattern in cell density is reflected in the dark adult stripes. Size-dependence, obtained through varying the incubation temperature, was studied experimentally, and it was demonstrated unequivocally that the length of the patterning domain at the time the pattern is laid down is crucial in determining stripe number, as we have learned to expect from the behaviour of lateral inhibition models, where the number of peaks depends on how many wavelengths can fit into the domain. The qualitative behaviour obtained from numerical simulations is consistent with the observed patterns.

A similar model has been proposed to account for many of the dramatic pigmentation patterns found on snakes [256]. Particular emphasis was placed in this application on the *growth* of the integument during the time the pattern is laid down. This matter was considered qualitatively in the previous alligator studies [253], where it was argued that embryonic growth on a time scale commensurate with the time required to lay down patterns could be responsible for the lighter shadow stripes observed on the ventral side of alligator bodies, between the distinct darker stripes on the dorsal side. In the context of snake patterns, this domain growth was studied as an integral part of the model, as a result of which spatially heterogeneous patterns considerably more complex than those found in other studies could be obtained numerically, including some of the complex multiple spot pattern elements observed on some snakes. A theoretical analysis specifically studying domain growth simultaneous with pattern generation [217] also found evidence for a surprisingly rich spectrum of complex spatial patterns in the simple cell chemotaxis model. Further analytical results described the propagation of spatially heterogeneous patterns, with estimates for the pattern wavelength and speed of spread [260].

The results obtained in the numerical simulations and theoretical analyses all demonstrate the considerable self-organizing potential of the chemotactic mechanism. They further strongly indicate that a study of domain growth simultaneous with the laying down of pattern would be rewarding for any of the models considered so far, in particular the reaction-diffusion and mechanochemical approaches, due to the remarkably novel behaviour observed in the chemotactic mechanism when growth of the integument was incorporated. Considerations of individual cell behaviour, when coupled with experimental interventions as begun in [253], appear to hold much promise for realistic applications of self-organizing models, to coat pattern formation and other developmental situations.

5.1.3 Cell-Cell Interactions

Mesenchymal cells may interact not only with some chemical species or the ECM, but also with each other (see section 2.1.3). Contact between cells gives rise to adhesion owing to interactions between cell surface molecules (possible effects of this are considered more fully in the context of epithelial cells which form extended cell sheets through cohering with each other — see section 5.3.1). Contact also results in the motion of mesenchymal cells being constrained when one cell meets a second, through contact inhibition of movement (CIM) — if the tip of a moving cell (the 'lamellipodium', or ruffled membrane, which is responsible for locomotion) makes contact with any part of another cell, the cell becomes quiescent, motion and ruffling ceases, and a little later another part of its membrane becomes active and the cell moves off in another direction. Such interactions may have morphogenetic implications, as they influence mesenchymal cell distributions and behaviour.

Cell Sorting

A significant example of this is cell sorting-out, illustrated through two classic experiments: the work of Wilson in 1907 demonstrated that cells from dissociated sponges, if allowed to re-associate, would spontaneously reform the original structure, with such re-aggregation being species-specific. Later experiments published by Townes and Holtfreter in 1955 showed that cells from different amphibian tissues would also sort out, so that the differences between cell membranes were tissue-specific as well as species-specific (for a discussion, see [16]).

The currently preferred explanation for sorting out is *differential adhesion* (see [332], with qualitative differences in adhesivity existing between the various cell types. The spontaneous reassociation of cells into structures after thorough mixing also constitutes a form of self-organization, but no quantitative model or explanation of this process appears to be extant (although simulations of cell sorting, based on the minimization of free energy, exist — see for example [118, 338], and section 5.3.1). As the scrambling of cells and juxtaposition of different cell types tends not to occur in normal development, the significance of the cell sorting mechanism is not clear, especially as many cell types or developmental stages do not display this property.

Cell Alignment

The cooperation between mesenchymal cells may lead to organization in another situation, however, for which more analyses are available. Interactions between the intrinsic properties of individual cells, as established *in vitro*, and the constraints imposed by the physical environment and the behaviour of other cells, can lead to relatively large-scale cellular organization through purely local interactions. In conjunction with contact inhibition of movement (CIM), which discourages end-to-end and end-to-side contacts between cells, associative movement (or *lateral adhesion*) encourages side-to-side adhesion between bipolar cells [16].

Fibroblast cells may move in various directions and reorient frequently and spontaneously, so that their motion consists of straight paths interspersed with occasional turns; but when a pair of cells come into contact, CIM comes into play. If the contact is however at a small angle, only a fraction of the lamellipodium is inhibited, and the contacting cell glides along its neighbour, realigns, and adheres to it. Generally fibroblast cells secrete collagen to form a matrix which they deform, and which guides motion, as discussed extensively above, in section 5.1.1; but it has been possible to observe the pure realignment phenomenon in experiments in tissue culture under conditions where the accumulation of collagen was inhibited. Thus haptotactic responses cannot occur, and the cells are free to move independently. After some time the formation of *parallel arrays*, or anisotropic patches of cells with a common orientation, is observed, so that cells spontaneously align. There is tug-and-pull competition between adjacent arrays, so that if the process is allowed to continue, eventually a single array with one axis emerges (for a general discussion, see [16]).

This behaviour has been studied in two distinct ways. Elsdale and Wasoff [95] were not concerned with the generation of the patterns, but rather with the classification of some of their features. They used analytical techniques such as geometric topology (unusually for a biological application!) to deal with the arrangement of discontinuities in the arrays, computing the topological index of pattern elements and hence characterizing the constraints on the possible types of pattern, with respect also to the boundary enclosing it. The heuristic arguments given, however, do no more than hint at the mechanism responsible for the origin of the fibroblast directionality and coherence.

Contact-mediated Formation of Parallel Arrays A rigorous mathematical model for the alignment of fibroblasts has been given by Edelstein-Keshet and Ermentrout [89, 90]. A feature of this model is that it demonstrates that direct, local cellular interactions and properties alone, not mediated by long-range chemical prepatterns or diffusional interactions, tractional forces, or effects of environmental structure, are sufficient for self-organization and the formation of patterns of aligned cells; no global organizing factors are needed. We thus have *short-range interactions* with the potential for *long-range correlations*, a feature of self-organization.

Various assumptions were incorporated into the modelling, including

- The direction of motion of the cell tends to persist;
- Cells make occasional random turns, with clockwise and anticlockwise turns being equally likely;

- Cells exhibit a contact response, by which they can turn and align with a contacted cell, and stick with it to become 'bound' cells forming clusters; and
- Cells can break off and leave clusters to become 'free' cells.

The formulation of the model is in the form of two integro-partial differential equations for the bound and free cell densities, initially treating only the way cells are distributed over different orientations, without spatial considerations. The detailed mathematical formulation is derived and analyzed in [90], and presented together with the results of numerical simulations.

Briefly, the model predicts that under certain conditions the numbers of cells recruited, and those lost from clusters, exactly balance so that a dynamic equilibrium between the numbers of free and bound cells at any orientation exists, giving a steady state solution; if all directions are equally likely, the steady state is uniform or homogeneous. In the presence of sporadic noise, the uniform steady state may be stable, so that cells remain randomly orientated; or it may be unstable, in which case favoured orientations are reinforced and parallel arrays form. As in other models, such self-organization is dependent on appropriate parameter values; in this case, for dominant orientations to appear it is necessary that:

- The cell population is large enough that encounter rates are sufficiently frequent;
- Clusters are cohesive;
- Cells stick to each other well; and
- Free cells do not get scrambled too rapidly by random turning.

As in other models, a characteristic 'spacing between peaks', whose size is determined by the parameters, also appears in the patterns; in this case it must be interpreted differently from usual, however, as parallel arrays are patterns in *angle*, not in *space*, so that there is a characteristic angular separation, not physical distance. Spontaneous cell alignment can occur through the variation of some bifurcation parameter through a critical value; for example, a critical cell density exists at which a sharp transition from random to preferred orientations occurs. The conditions for patterning are here, and in [89], described heuristically, but quantified rigorously in [90].

The above patterning is, as already stated, merely an angular distribution; the full spatial behaviour is extremely complex, and has thus not been treated analytically, only simulated using cellular automata (see also [97]). This modelling technique, to be discussed more fully in the next chapter (see section 6.2), is computationally convenient, and enables the time development of the spatial distribution of oriented cells to be followed visually. The observed behaviour is as expected; stable parallel arrays form if the total number of cells is high enough, whereas if there are insufficient cells, no orientation eventually wins out. The simulations hence confirm the self-organizing behaviour arising from simple contact interactions, not dictated by any structural constraints, interactions with boundaries or other direction inducing effects.

The authors suggest [89] that, whereas here cellular orientation is the property to be organized, similar models could address the organization of other cellular properties in which the state of a cell may be directly influenced by interactions with its neighbours: these possibly include degree of electrical activity, phase of the cell in its cycle, metabolic state or gene expression.

Hence a powerful general framework for local self-organization, not mediated by any chemical or mechanical intermediates, has been established, which may be generalized and extended to other analogous situations.

5.1.4 Neural Models

"Perhaps the most obvious, ubiquitous, important and complex spatial patterning processes are those associated with the nervous system, such as pattern recognition and the transmission of visual information to the brain" [251, p.481]; and the development of this intricate functional structure is similarly highly complex. It is largely for this reason that we do not in this work consider neural development and patterning (see section 2.3.2). As the nervous system develops rather late, it also plays no role in the major patterning events we have studied, occurring early in embryogenesis. The vast field of neural patterning should, however, at least be touched upon, not least because the concept of lateral inhibition arose from visual recognition studies, in the visual illusion known as *Mach bands* (for a discussion, see [288]). In a neural net, for example in the retina, an excited ganglion cell inhibits the activity of its neighbours; this behaviour is 'hard-wired' as inhibitory neural connections. The local activation appears as the stimulus triggering neuronal firing. In the light of our experience with lateral inhibition models, we would expect extensive pattern-forming potential in neural models, as is indeed the case.

A Neural Model for Molluscan Pigmentation

Neural models involve also the interactions of individual cells, justifying their present categorization under individual cell behaviour, although they are not associated with the distribution in space or orientation of motile cell distributions as in the models above; rather, patterns of excitation are generated, with the dependent variable being for example the firing rate of cells. Of interest to us here is the neural model of Ermentrout and coworkers [96], in which a variety of pigmentation patterns on mollusc shells could be reproduced. The model assumes that the mantle cells that secrete the pigment, which is deposited at the growing edge of the mantle, are driven by stimuli from a neural net with the property of LALI (see [287]). The mathematical formulation combines discrete time models with continuous spatial variation, based on three assumptions:

- Cells at the mantle edge secrete material intermittently, corresponding to the discrete time intervals in the model;
- The secretion during a given period depends on two factors, the neural stimulation from surrounding regions of the mantle, and the accumulation of an inhibitory substance present in the secretory cell;
- The net neural stimulation of the secretory cells comprises the difference between the excitatory and inhibitory inputs from the surrounding tissue.

These conditions are incorporated into a model formulation of four equations, two governing the amount of secreted pigment and the amount of a refractory substance produced during secretion in the time period under consideration, and two integral equations to account for the

excitation and inhibition which generate the lateral inhibitory field in the neural net; for the complete formulation and analysis, see the original paper [96] or the extensive discussion in [251]. The analysis and numerical simulations reveal a bifurcation from the uniform solution to spatial patterning, that is again self-organization through symmetry-breaking. Depending on the parameter values, diverse patterns such as vertical, horizontal and diagonal stripes, wavy stripes, checks, irregular stripe patterns, tents and so on, corresponding well to many of the complex and beautiful spatial patterns observed on mollusc shells, could be generated.

Comparison with a Reaction-Diffusion Approach We have previously noted the model of Meinhardt and Klingler for shell patterning, based on a reaction-diffusion approach [236] (see section 4.3.2). We are thus faced with two models for the same situation, and which make qualitatively very similar patterning predictions in the range and realism of the patterns they can reproduce. The similarity in predictions is a manifestation of the underlying equivalence of lateral inhibition models, as discussed in [287, 288].

The models, however, suggest quite different experimental interventions to test their assumptions; the neural model suggests administering neuroactive drugs, while a diffusion-based model predicts that patterning should cease in the presence of barriers to diffusion. Unfortunately, it turns out that almost any intervention causes the mollusc to cease its shell-depositing activity, so that appropriate experiments, though obvious in principle, are difficult in practice. Nevertheless, the situation of yet another example of a model for integumental patterning, displaying essentially equivalent self-organizing properties from very different assumptions, is instructive.

We have considered a variety of situations in which individual cells, interacting with each other, with attracting chemicals or the extracellular matrix, play an integral role in their own patterning. The motion, properties and interactions of mesenchymal and neural cells can thus form spatially heterogeneous distributions through a self-organizing process, well understood in the context of lateral inhibition. We next consider how *intra*cellular properties can lead to patterning in the individual cell and hence possibly to more global morphogenesis.

The level of discussion of the rest of this chapter, on epithelial morphogenesis, will be less mathematical and more biological, compared with the aforegoing. This is because increasing biological detail correlates with mathematical formulations that are too intricate to consider here; this will especially be the case for the models in the next section 5.2. Thus for this section (and indeed for the remainder of the thesis) no more equations will be presented; rather, the assumptions and results of the models and their relations to each other will be described and their significance assessed, while the model formulations may be found in the original publications.

5.2 Intracellular Properties and their Influence on Epithelial Morphogenesis

In the previous section we studied the potential self-organizing properties of mesenchymal cells, individual cells which may cooperate to generate coordinated collective behaviour. In the process we considered largely intercellular interactions, and paid no significant attention to the internal properties of cells, except insofar as these could be modelled as measurable, macroscopic inter-

cellular interactions. Embryonic cells form two major groups, and as already discussed in section 2.1.3, epithelial cells display quite different features from mesenchyme (although the distinction is not rigid — a conversion of cells from epithelium to mesenchyme or *vice versa* occurs at several stages in development, for instance in the formation of nephrons, indicating that the cell types possess largely the same intracellular, cytoskeletal machinery).

Epithelial cells form, usually monolayered, sheets, and this defines and constrains their behaviour; so that the morphogenetic properties of epithelium must always be considered in terms of the behaviour of the whole sheet. The layers of cells, however, display a wide repertoire of behaviours, being able to move, and change shape both through the deformation of individual cells and through the rolling, buckling, folding or invagination of the entire sheet, which still retains its integrity as a connected tissue. A significant feature of epithelial cells is their polarity: Each cell, and hence the sheet, has a basal surface which is usually anchored to a basal lamina or other extracellular matrix material, and an apical surface which remains free, and faces the external medium or an internal cavity. The requirement to maintain a free surface significantly restricts, on topological grounds, the range of forms that epithelia can take up.

There are two major approaches to the study of epithelial morphogenesis, corresponding essentially to holistic and reductionistic viewpoints. The cell sheet may be considered as an integrated unit, and continuum models of the deformations of sheets with certain properties may be studied (the 'whole tissue' approach); or the deformations and shape changes of individual cells may be modelled, and these used to predict the macroscopic behaviour of the entire sheet. Ultimately, of course, a unified approach is required. We will proceed in this section 5.2 in a more reductionist vein, and consider the mechanochemical properties of individual cells, and how these influence cell shape and behaviour. Our focus will be directed firstly at individual cells and the patterning of some unicellular organisms, and will then continue to the effect the properties and deformations of individual epithelial cells have on the entire cell sheet.

5.2.1 The Mechanochemistry of Cytogels

There has recently been much experimental and theoretical interest in the spatial and temporal behaviour of calcium inside cells, as evidenced for example by the publication of the recently introduced journal, *Cell Calcium*. We have already noted (see section 4.3.4) the status of calcium as a potential morphogen in the marine alga, *Acetabularia*, to which we shall return later. Of interest here is the potentially wide general applicability of the coupling between calcium and the viscoelastic properties of the cell cytoplasm, the interior of the cell. This may provide an explanation for the contractility and deformations of the individual cells; and hence, in the case of epithelial cells, for the consequent morphogenetic movements and deformations of cell sheets.

This study of the influence of calcium in morphogenetic processes is based on strong experimental evidence, in conjunction with modelling. The primary morphogenetic events are in this case due to physical forces largely mediated by the cytoskeleton, and spatio-temporal variations in regulatory species, such as calcium. Again, as for the models for mesenchymal morphogenesis above, there is direct coupling between patterning and morphogenesis, thus obviating the need to invoke the hypothetical morphogens and interpretatory mechanisms which are implicit in prepattern theories.

Fairly extensive models for the mechanochemistry of cytogels have been developed, all of

which display a degree of complexity considerably exceeding any of the previously-considered models. In spite of considerable simplifications, this is an inevitable consequence of any attempt at realistically capturing the relevant molecular features. Thus we shall not attempt here to present any of the equations or detailed analyses, but just sketch the basic assumptions and major pertinent results very briefly, referring the reader to the original publications for further details. We further note that there have been two slightly different major approaches, one due to Oster and his coworkers (see [251, 294]), and the other introduced by Goodwin and Trainor [131] and subsequently expanded.

The Molecular Basis of the Cytogel Models

The molecular constitution of the cytoplasm is highly complex, but some salient features may be identified (see also section 2.1.2, and [131, 251, 294, 353]): It consists largely of a viscoelastic gel, which is a network of macromolecular fibres mainly composed of the protein actin linked by myosin crossbridges; the contractile activity thus occurs in a manner analogous to muscle contraction. The network is a dynamic structure, and the assembly and disassembly of the crosslinking of fibres, combined with shape changes, occurs actively. When the fibres are strongly linked, the cytoplasm tends to gel, while it solates (becomes more fluid) with weak links.

This sol-gel transition or degree of actomyosin crosslinking depends on several factors, but mainly on the local concentration ratio of free to bound *calcium* in the cytogel, which regulates the contractile machinery by affecting the viscoelastic properties of the medium, inducing conformational changes in the cytoskeleton and associated proteins. At low concentration levels, calcium encourages crosslinking, so that fibres tend to shorten and hence get stronger. Hence as free calcium goes up the gel first begins to contract actively. If the concentration gets too high, however, the gel becomes solated and can no longer support any stress. There is thus a 'window', an ideal range, of calcium concentration, associated with optimal contractile activity; this immediately indicates a nonlinear dependence of the dynamics on the calcium concentration.

The calcium in the cytogel is sequestered in membranous vesicles, and may be released through an autocatalytic process, known as calcium-induced calcium release (CICR). That is, if the free calcium concentration exceeds a certain threshold value, it can trigger a local autocalytic cascade release of calcium, for instance through the action of calcium-activated enzymes such as gelsolin. The act of straining the cytogel may also mechanically trigger the release of sequestered calcium, through 'stretch activation', by initiating a membrane depolarization of the sequestering vesicles.

The above comparatively intricate biochemical details form a minimal summary of the underlying assumptions that need to be incorporated into any realistic model of intracellular deformations; they have been included as an indication of quite how complex any attempt at realistic biological modelling inevitably is. For a more detailed discussion of the relevant features of the mechanochemistry of the cytogel, see [294], where the basic assumptions of a model about cytoplasmic contractility are summarized as follows:

- Ca^{2+} levels regulate the sol-gel equilibrium of the cytoplasm; that is, the number of actin-actin cross-links as well as the average lengths of the actin filaments.
- Ca^{2+} initiates active contraction of the actomyosin gel.

- Calcium is sequestered locally in membranous vesicles.
- An autocatalytic release of Ca^{2+} from these vesicles can be stimulated by raising local Ca^{2+} levels above a threshold value.
- Release can also be stimulated by mechanically straining the cytogel.

Models of Cytogel Behaviour The object of any modelling is to describe a possible scenario for how the calcium-coupled viscoelastic activity in the cortex translates into patterns, deformations of the cytoplasm, or motion of the cell, depending on the application. To this end, model equations are set up, which in the simplest case describe the mechanical equilibrium, or *force balance equation*, of the cell cortex or cytogel, modelled as a homogeneous viscoelastic medium dynamically coupled to the calcium ion concentration; and the *calcium conservation equation*. Modelling the cytogel as a single homogeneous medium is a drastic compromise in view of its complex composition; more recent work has weakened this assumption somewhat and incorporated the study of the alignment of intracellular actin filaments in response to anisotropic stress [321]. But even with these simplifications — and note that a realistic description would surely require the inclusion of regulatory factors other than calcium, and the independent consideration of different structural components of the cytoplasm — the constitutive equations are quite detailed and intricate (see especially the mechanical equation (1) for the local displacement field derived in Goodwin and Trainor [131]), and well beyond analytical tractability.

The model mechanical and calcium conservation equations (literature references to specific forms of these will be given in the discussion of applications) have also been extended to include effects such as calcium diffusion, input or output; separate consideration of the gel and sol quantities; tractional forces and osmotic pressures; and possible dependencies of any or all parameters on calcium concentration, stress or dilation, or external factors. Clearly, more realistic modelling would incorporate a diversity of other effects, communication and coupling between cells, epithelial-mesenchymal interactions, and so on — the possibilities are essentially 'endless': unfortunately increased realism invariably goes hand in hand with decreased analytical and numerical tractability, and consequently usefulness of the model. Thus it is appropriate now, rather than dwelling longer on forms of intracellular calcium-based model equations, to consider some of the applications that have stimulated this line of mechanochemical research.

5.2.2 Tip and Whorl Morphogenesis in *Acetabularia* — the Work of Goodwin and Trainor

We have already considered the work of Harrison [152] and others on the regeneration of the tip of the unicellular marine alga *Acetabularia mediterranea*, on the basis of a kinetic, reaction-diffusion scheme; see section 4.3.4. Extensive evidence was presented in favour of such an approach, including:

- The Arrhenius-like temperature-dependent spacing between the hairs in a whorl [155], which would be consistent with some form of chemical kinetics;
- The dependence of spacing on external calcium concentration, implying some morphogen-like role for this ion; and

- The otherwise invariant spacing over a range of domain sizes (so that the number of hairs varied considerably with tip circumference) as would be predicted through the chemical wavelength arising from reaction-diffusion models.

But we have already noted in our consideration of other self-organizing mechanisms that the above features are generally found in the solutions of models that display lateral inhibition and symmetry-breaking properties, rather than being restricted to reaction-diffusion systems *per se*, on account of the frequently similar mathematical formulation. This vindicates the study of other schemes for *Acetabularia* regeneration, and in particular a mechanochemical theory in terms of the interaction of the cellular cortex and intracellular calcium, proposed by Goodwin and Trainor [131].

The Goodwin-Trainor Model

The original model [131] considered the nature of the spatial ordering which arises from calcium regulation of the viscoelastic properties of the cell cortex, in the general manner described above, section 5.2.1. Thus, on the basis of the experimental evidence for the influence of calcium on the physical state of the cytoplasm, equations for the changes in calcium distribution as a result of mechanical perturbations, and the effects of calcium on the mechanical state of the cytoskeleton (which was assumed to be a linear viscoelastic homogeneous medium) were derived; the equation for the local displacement field $\xi(\mathbf{x}, t)$ is especially complicated, incorporating a range of elastic and viscous effects [131].

In the original study, simplified linearized equations were solved numerically, and the potential for spatial instabilities demonstrated. This was considered as circumstantial evidence that symmetry-breaking and self-organization could produce the spatial morphologies corresponding, for example, to whorl formation, with the patterns being manifested possibly as inhomogeneous viscoelastic strain fields which were assumed to couple to the cell wall and induce the overt external structures. The immediate continuation of these studies was the full linear stability analysis of the original Goodwin-Trainor equations for the viscoelastic properties of cytogel [353]. This work showed how, in an isotropic approximation, we effectively need consider only a one-dimensional problem for instability; whereas in the anisotropic case, coupling occurs between compressional and shear modes.

Extensions to the Basic Model Further work, in particular by Goodwin and coworkers, extended the original 'GT' equations by incorporating some other effects. The morphological changes we wish to consider, of the formation of the tip and whorls of hairs, lie at the level of cell wall deformations, arising from the pressure-induced strain field acting on the cell wall and the cytoskeleton. Thus Brière and Goodwin [38] studied axially symmetric solutions of the Goodwin-Trainor equations on two-dimensional surfaces, considering the elastic equilibrium of the cell wall, in order to establish the relationship between the actual geometry of the regenerating tip and the predictions of the model; they found that the first stages of apical regeneration of *Acetabularia* could indeed be simulated by the mechanochemical model when solved on a realistic domain. An extension, using membrane theory to consider the deformations of the cell wall primarily, rather than as a secondary effect of the strain inhomogeneities of the cell cortex, has been made by Chaplain and Sleeman [55]. They showed that when the cell wall is treated

as a membrane subject to an internal pressure, and the tip growth simulated using a moving-boundary formulation, with cytosolic free calcium concentration and turgor pressure being the two variables responsible for growth, the resultant equilibrium configuration is consistent with the observed shape of the tip in *Acetabularia* and the predictions of the previous work.

Brière and Goodwin [39] further extended the model to consider calcium input and output processes, and showed that all previous predictions were robust with respect to calcium fluxes through the plasma membrane or calcium exchanges with cytoplasmic vesicles; perturbations of the homogeneous equilibrium would still be driven unstable by the basic calcium-strain interaction. Thus various calcium exchange and storage processes could be added to the simple mechanochemical coupling with no loss of self-organizing potential; applications to *Dictyostelium*, *Drosophila* and *Acetabularia* were discussed. The effects of diffusion were considered by Hart, Trainor and Goodwin [158], who generalized the basic equations to the case where the concentration of (free and bound) calcium ions was allowed to change locally; stability analyses again confirmed that for appropriate parameter values intermediate wavelength perturbations may become unstable.

Lessons from the Goodwin-Trainor Model The analyses of the Goodwin-Trainor model, applied especially to tip and hair morphogenesis in *Acetabularia*, provide an instructive example of the formulation and analysis of a self-organization model: A basic, minimal model is proposed on the basis of experimental evidence, and analysed in the light of a plausible application. Extensions of the model to incorporate further potentially important effects are studied (some examples of these effects are given above) and shown not to require significant modifications to the model predictions. Thus a process of successive refinement, always keeping the intended experimental application in mind, is displayed, which is a hallmark of good modelling. Finally, we are faced with a conflicting theory, the reaction-diffusion approach; thus each protagonist compares the models [131, 150] and notes that at present there is no compelling evidence to choose between them, but that extensive work is being carried out to design appropriate experiments to discriminate between the reaction-diffusion and mechanochemical paradigms [151].

The Goodwin-Trainor model is specifically concerned with the morphogenetic implications of intracellular behaviour on the patterning of a single cell, as it treats mainly a unicellular organism. As we shall see, however, similar considerations of single cell deformations may have extensive implications for the behaviour of cell sheets and structures.

5.2.3 The Work of Oster and Collaborators

Oster, in conjunction with Odell, Murray and other coworkers, has developed a range of applications of the theoretical considerations on the mechanochemistry of cytogels. The work is again based on the general model formulations outlined in section 5.2.1 (although the detailed assumptions and equation formulations used differ somewhat from those of Goodwin and Trainor). A wide range of applications, not restricted only to pattern formation and morphogenesis, has been treated by Oster, with implications both for the behaviour of individual cells and cell sheets.

A minimal model for cytogel patterning has been proposed by Murray and Oster [258], which considers the basic biological facts about cytogel as presented above, to derive a force balance equation for cytogel contractility, and a calcium conservation equation. A linear stability anal-

ysis demonstrates the potential for instability and spatial pattern generation. One-dimensional travelling wave solutions to the equations, as well as surface waves on a spherical geometry, simulating deformation waves such as post-fertilization waves on vertebrate eggs, have also been demonstrated; see [251] for a discussion. A more general discussion of the mechanochemistry of cytogels has been given by Oster and Odell [294], in the general vein described above, but incorporating also conservation equations for the actomyosin monomers and fibres. The general formulation is far too complex for a detailed analysis, but interesting specializations of the model, corresponding to particular biological situations, may be obtained, in the three general areas of spatial patterns, wave phenomena and oscillations.

A Model for Cell Motility The physical chemistry of actomyosin gels has been used, for example, to investigate cell motility [295], for which the postulated biological mechanism will be described here briefly: Cells moving *in vitro* do so by spreading a broad, flat sheet of cytogel, the lamellipod, in front of the cell to pull the cell forward. It is assumed that the actomyosin cortical gel undergoes a cycle of events involving solation and osmotic expansion, followed by regelation and contraction. The solation is triggered by an ionic leak, which may be due to the binding of a chemotactic agent on the membrane, or to a depolarization of the cathode-facing membrane in a galvanotactic mechanism. Owing to the loss of ions, the gel network is partially degraded, relaxing its elastic modulus and allowing it to expand osmotically under the influence of the cell's internal swelling pressure; this extends the leading edge of the lamellipod. The tip adheres to adjacent cells or the substrate due to adhesive molecules carried by the flow of the cytoplasm to the tip, which anchors the cell. Thus when the cortical actin regels, active contraction occurs and the cell inches forward, provided the contraction is sufficient to break the posterior attachments of the cell to other cells or substrate. The cycle can reinitiate once the gel has relaxed to its initial state.

The mechanism for cell motion here described heuristically has been given a rigorous mathematical formulation [295] in terms of the mechanochemical model for the cytogel. This example indicates the range of applications, not just involving pattern formation, that may be considered in terms of model equations displaying self-organizing properties.

Oscillations in *Physarum* Another situation, in which *temporal* self-organization is displayed, is the rhythmic mechanical contractions and oscillatory 'shuttle streaming' of the plasmodial strands of the slime mould *Physarum*, which have also been modelled on the basis of the general model of the contractile properties of actomyosin gels [293]. The model domain for plasmodial oscillations consists of a hollow cytogel cylinder filled with solated cytoplasm; the model equations are highly complex, but it was possible to show that if the kinetics are in an excitable regime, the model plasmodium will exhibit rhythmic contractions driving a periodic fluid flow in the core of the cylinder. In fact, as parameters are varied, a single limit cycle generated by a Hopf bifurcation could be shown to cleave into two limit cycles, indicating complex temporal behaviour, corresponding to experimentally observed plasmodial dynamics. Again the self-organizing behaviour of a single cell may be described by the mechanochemical approach.

Spatial Patterning — the Formation of Microvilli It is however in spatial pattern formation that we are most interested, and with particular reference to epithelium. Before we

proceed to consider the effect of intracellular behaviour on deformations of cell sheets (see the next section 5.2.4), we consider spatial patterns on the epithelial cells themselves; for example, the formation of microvilli on the cell surface, foldings on the cell membrane in a regular hexagonal array. A model for their formation has been proposed by Oster, Murray and Odell [291], who show how calcium-triggered contractions of the actin cortex can lead to regular spatial arrays. That is, a uniform density distribution of actin is spatially unstable, and the observed patterns are essentially the hexagonally periodic solutions for the tension patterns which are generated. Thus arrays of spaces less dense in actomyosin are created. Osmotic pressure then expands these regions outwards to initiate the microvilli. The complete model consists of conservation equations for the gel, sol and calcium, combined with a force-balance term which incorporates elasticity, viscosity, active stress and osmotic pressure; linearizations demonstrate the possibility of spatially inhomogeneous solutions. The hexagonal patterns correspond either to hexagonally symmetric solutions in two dimensions, or to the sequential formation, row by row, of interdigitating one-dimensional patterns, as for the formation of feather germs above in section 5.1.1.

The motivation behind the above approach is similar to that we have previously encountered in the study of *Acetabularia*: instabilities of the calcium–gel coupled system lead to spatial pattern formation in the cytogel itself, which manifests itself in inhomogeneities in the cell cortex and hence in protuberances from the surface. A similar mechanism may underlie other structures, such as the pattern of tentacles on hydra.

The understanding of developmental mechanisms profits greatly from the elucidation of mechanisms at work in such relatively simple single cell systems, but the ultimate goal is to appreciate the processes at work in multicellular eucaryotic organisms, and the coordinated patterning of *assemblies* of cells. To this end, we now proceed to seek an understanding of the deformations of *cell sheets* in terms of the shape changes arising in the individual cell, as modelled for example by the above mechanochemical calcium ion/viscoelastic gel coupling.

5.2.4 Deformations of Epithelial Sheets

An epithelial layer as a whole can deform by spreading, retracting, folding, bending, rolling into a tube, evaginating or invaginating [16]; these deformations are accompanied, and indeed driven, by shape changes of the constituent cells. Hence epithelial morphogenesis may be considered in terms of the question of how the *local* cell shape changes are orchestrated into *global* morphogenetic movements and deformations. Such considerations indeed motivated the first studies of the mechanical basis of morphogenesis.

Global Deformations through Local Cellular Instabilities

A discrete model for morphogenetic folding of embryonic epithelia was presented by Odell and coworkers in a landmark paper [283]. In that work, the cytoskeletal properties of cuboidal epithelium were considered; in particular, the cells were presumed joined at their apices by circumferential junctions, to which were attached arrays of microfilament bundles. The contraction of these bundles acts in the manner of a 'purse string', and constricts the apical surfaces of the cells. As the cells are considered to have constant volume, such apical constriction causes the cells to elongate at their bases and become *wedge-shaped*, with a larger basal than apical surface

area. Such contraction, spread over the entire sheet of cells joined together, introduces a bending moment or torque within the cell sheet, which can produce global deformations such as buckling or folding of the epithelium.

In the model of Odell *et al.*, the microfilament bundles are assumed to be *excitable*: if they are stretched beyond a certain threshold, an active contraction is triggered which 'draws the purse string' and reduces the apical circumference of the cell to a new, shorter resting length. With the apical coupling between cells, contraction in one cell stretches the next, and in turn initiates 'firing' and contraction if the threshold length is exceeded; thus a *wave of contraction* is propagated over the surface of the epithelial sheet. Thus a global deformation requires only an array of passive cells and one active (triggerable) element at the apical end. Once this cell is triggered, the process of contraction and deformation is initiated.

In the formulation of the model [283], each microfilament bundle is represented as a viscoelastic 'dashpot' element, and a mechanical model of the cytoskeletal interior of the cell is built up as a quadrilateral (in two dimensions) each of whose sides and diagonals is such a viscoelastic unit, but only the apical end of which is mechanically excitable. The complete ring of cells is obtained by coupling these discrete cellular elements, resulting in a finite element formulation for the complete model. Important to the formulation is that due to the high viscosity, inertial forces are negligible on the embryonic scale and may essentially be ignored in the equations of motion.

Using this model formulation with different model parameters, computer simulations were able to reproduce many of the features of the folding and rolling movements associated with gastrulation, neurulation, ventral furrow formation and other processes. As Bard [16] points out, the evidence to support a model based on triggered contraction is not substantial, but the remarkable likeness of the computer-generated simulations, especially to the processes of gastrulation and neurulation, does provide considerable circumstantial encouragement for believing such a mechanism; and the simulations generated by the model have been much cited, even in more popular works (see for example [378]). The crucial aspect, of course, is that once triggered, the morphogenetic deformations proceed *autonomously*, directed solely by the global balance of forces and the local cellular properties, with no need to invoke special individually programmed sequences or patterns of cell shape change.

Continuum Modelling of Individual Cells The model of Odell *et al.* [283] is based on discrete viscoelastic elements coupled in the individual cellular structure, with each cell then forming a finite element in the overall array. These studies, in which it was assumed that cells trigger one another's contraction by mechanical activation, may also be extended to incorporate chemical coupling by transport through gap junctions. Clearly, the array of cells constituting the epithelium may also be treated using the continuum model for the deformation of cells through the mechanochemical interaction between calcium and the viscoelastic cytogel, discussed at some length in the previous sections, with similar predictions [251, 294]. Indeed, the finite element formulation may be seen as a discretization of the continuum model. Thus local cellular deformations may lead to global patterning.

This behaviour appears to be self-organizing only in a limited sense, however: there is the instability of the individual cell which, once triggered, initiates a spontaneous deformation and symmetry-breaking. On the global scale, however, the final shape is dictated by the contraints of

mechanical equilibrium, and is an inevitable, deterministic consequence of the local deformations. A local departure from equilibrium leads to a new, deformed, global equilibrium configuration.

The 'Cortical Tractor'

A more recent approach to epithelial deformation is the 'cortical tractor' mechanism proposed by Jacobson and coworkers [175]. This model similarly assumes the interaction of epithelial cells, joined at their apices, through their cortical activity. More particularly, it is assumed that there is a constant streaming of cortical cytoplasm, flowing from the basal and lateral surfaces to the apical region, and that associated with this flow is a continuous removal and replacement of cell surface molecules. The apical seal that characterizes epithelial sheets is thus a dynamic structure, in constant molecular flux, due to this putative *tractoring* mechanism. This constant creation and recreation of the membrane allows it to be sufficiently fluid for cells to move past one another without tearing their surfaces, thus providing a mechanism for active rearrangements within an epithelial sheet; and the energy imparted to the membrane can, when accompanied by a gradient in the tractoring activity, lead to deformations in the cell sheet. This is through the generation of shear forces between cells 'crawling' at different intensities; the apical junctions restrain cells from detaching from the epithelial sheet, so that the net effect of differential crawling is a deformation of cuboidal epithelial cells to columnar morphology and a folding of the epithelial sheet.

Again, the verbal arguments for the feasibility of such a tractoring process [175] are buttressed by a mathematically-formulated mechanical model, the derivation and analysis of which are given by Cheng *et al.* [56]. Computer simulations are demonstrated that reproduce the main features of neurulation, including the shape changes of the plate cells, the shrinking of the total surface area of the neural plate, the systematic neighbour changes (*interdigitation*) of the notoplate cells, the creation and elongation of the neural folds, and the rolling up of the plate into a tube. (The sequence of cell shape changes and deformations required for the complex prcess of neurulation was previously elucidated by Jacobson and Gordon [174] through extensive experimental and mathematical studies and computer simulations.) In fact, the cortical tractor can account for all the epithelial foldings simulated by Odell *et al.* [283].

Assessment of the Cortical Tractor Mechanism The cortical tractor model is very complex, and lacks the crucial experimental backup which would give it full weight, as the process of tractoring, depending on constant replacement of membrane molecules, while certainly possible or even likely in view of the dynamic nature of cells, does seem rather energetically expensive [16]. On the other hand, any model that goes some way towards accounting for the complex and little-understood process of neurulation should not be taken too lightly. More evidence is needed before a balanced assessment can be made.

This mechanism postulates differential tractoring to produce epithelial deformations such as neurulation, so that an initial gradient, such as an ionic gradient across the neural plate, is required. Such a gradient could be generated by one of the means discussed in the previous chapter, but the requirement for an initial inhomogeneity rules out strict self-organization for the tractoring mechanism. Nevertheless, once begun, the mechanism proceeds autonomously to generate novel structure.

The Formation of Placodes In view of our extensive discussion of the formation of dermal papillae, the mesenchymal aggregations that form feather primordia (see section 5.1.1), it is appropriate to note briefly that the individual viscoelastic properties of cytogels, when considered in the context of an epidermal layer of epithelial cells, can account for the formation of periodic thickenings of the epidermis, or papillae, which overlie the placodes [294]. A finite element model of an epidermal layer of cells attached to an elastic substratum has been used to model this situation. When the cellular array is triggered at one end, two waves sweep over it: a fast mechanical stress wave followed by a slower trigger chemical wave. Following the passage of the waves, the epidermal cells are fired, and thickened arrays of columnar cells, or placodes, begin to form spontaneously. We noted above that evidence from dermal-epidermal recombination experiments points to the epidermis as determining the patterning of primordia [16]; this model may provide a mechanochemical basis for such epidermal patterning.

We have considered fairly extensively the intracellular properties of individual cells, and their consequences both for the patterning of single cells and the deformations of cell sheets. Epithelial deformations may however also be treated on a more holistic basis, in terms of global properties of sheets considered as a continuum, rather than by a discretization in terms of individual cells. This will be the subject of our final major section of self-organizing properties in development in a dynamical systems, differential equations approach.

5.3 Surface Deformations in Epithelial Morphogenesis: Global Properties

In the previous section, we explicitly studied the *intra*cellular interactions leading to deformations of individual cells, and the effects of these shape changes on the overall behaviour of epithelia. Several authors have taken the alternative approach of treating the global behaviour of the cell sheet treated as a continuum. Thus the discrete cellular composition of the tissue is (temporarily) neglected, except possibly to incorporate into the modelling general tissue properties abstracted from known characteristics of individual cells.

On the whole, the motivations and methods of these studies are quite different from those we have considered above. For instance, while frequent attempts are made to relate the proposed models to biological experiment, and provide a molecular basis for the assumptions, the variables considered in the models tend to be largely abstract and phenomenological. Also, the intention is usually to account for observed surface deformations in terms of *equilibrium* considerations, often involving energy minimization; it is generally shown that a given configuration of, especially, adhesivity or surface tension will under appropriate conditions autonomously lead to a shape change not unlike one observed in development. It is indeed a necessary and nontrivial problem to demonstrate that, say, the internal surface forces arising from a given distribution of adhesion molecules are sufficient to account for a certain tissue shape, without requiring any additional external forces or further information input in the form of, for instance, genetic instructions directing cell-autonomous behaviour.

Nevertheless, on the whole this modelling does not deal strictly with self-organization *per se*. That is, a previous inhomogeneity, in the form for instance of a pattern of adhesion molecules

or an initial appropriately situated minor buckling of the cell sheet, is needed; studies focussing on symmetry-breaking would need to concentrate on the *origin* of this previous inhomogeneity, which constitutes a form of prepattern, or positional information. Clearly, new and novel structures form, through the action of mechanical forces on the cell sheet, but these manifest the inexorable tendency towards equilibrium, whereas self-organization, as we saw in chapter 3, constitutes a specifically nonequilibrium phenomenon. Thus we will not be concerned much with these models for epithelial morphogenesis, merely pointing out some of the main aspects that have been treated.

5.3.1 Adhesion and Surface Tension

Certain physical properties well known from non-living systems act also upon the embryo, and play an important role in guiding and constraining the types of processes that may occur in development, overlaying the genetically specified developmental processes by an epigenetic influence. This fact should be intuitively obvious, especially to anyone with an inclination towards physics, but has frequently been neglected by those who would seek to account for biological processes solely in terms of biochemical interactions and gene expression patterns. Throughout this thesis the expediency of a more physical approach has been apparent, from the consideration of reaction-diffusion equations borrowed from chemical kinetics (see for instance the BZ reaction) to mechanical forces, and even the influence of gravity on the polarity of the *Xenopus* egg. Indeed, the fundamental paradigm of self-organization has ultimately been derived from physical (and mathematical) considerations — see chapter 3.

The general review by Newman and Comper [268] draws particular attention to 'generic', broadly applicable physical mechanisms, as opposed to 'genetic' factors. In this work, they stress especially the significance of *interfacial tension* and *adhesive differences* in the patterning of epithelia. The form of a population of adhesive cells will largely be determined by the drive towards energy minimization. Thus, for example, a homogeneous isotropic population of cells will tend towards spherical form. Anisotropies in cell surface adhesion will determine other most-stable, or equilibrium, configurations, such as cell sheets, tubes or vesicles [332]. The molecular basis of adhesion, predominantly based on the class of cell adhesion molecules (CAMs) [82, 84], has already been considered (see section 2.1.2). For instance, varying CAM expression patterns will tend to cause localized clumping, which could be a manifestation of an underlying prepattern; such a model has been proposed for somitogenesis [15].

Adhesion and Mesenchymal Cells We have already noted the classic sorting out experiments of Holtfreter (see section 5.1.3) in which dissociated mesenchymal cells were able to reaggregate into their original tissues. The most commonly accepted explanation for this is the *differential adhesion hypothesis* advocated by Steinberg (see [332]): motile, adhesive cells will naturally tend to group so as to maximize their adhesive interactions, as this minimizes interfacial free energy. Thus heterogeneous cell populations such as those studied by Wilson (sponge cells from different species) and Townes and Holtfreter (different amphibian embryonic tissues) may preferentially either intermix or sort out, depending upon the balance of adhesive forces between like and unlike elements; the precise configuration adopted depends on the particular adhesive relationships which prevail.

The morphogenetic behaviour of tissues may be accounted for by specific analogy to the behaviour of liquid droplets [268]: tissue fragments can flow in response to external forces and coalesce with other tissues, much like drops, and the final configuration after separation of mixtures of cells from different tissues is just as would be predicted if the tissues, like simple liquids, exhibit interfacial tensions with respect to their surroundings. The characteristic interfacial tension between two immiscible liquids depends on the area of the interface; so that the interface will have a well-defined shape in order to minimize the area and hence the total free energy, consistent with all internal and external constraints. Such considerations dictate the spreading, adhesion or deadhesion, and deformation of cell sheets. Thus much morphogenetic behaviour is analogous to equilibrium phase separation. Sulsky *et al.* [338] present a simulation of cell sorting based specifically on differential adhesion; a further discrete simulation of such a sorting out effect with application to animal coat patterns will be discussed later (section 6.2.2). The potential morphogenetic influence on mesenchyme of haptotaxis, due to a gradient in adhesivity, has also been noted.

Adhesion in Epithelial Deformations

Concerning epithelial adhesion-driven deformations, an approach to the analysis of cell shape and intercellular adhesion has been given by Stein and Gordon [331], who model epithelia as 'bubble rafts'. They provide a computational method, based on the liquid analogy, for approximating the shapes of cells and calculating the resulting gradients of surface tension in epithelia. This is intended mainly as an algorithm to aid future work, but Chichilnisky [57] uses a similar approach to the differential adhesion between cells, this time based on a generalization of the equations describing soap bubbles, to predict explicitly the energy-minimizing configuration of monolayer epithelia, based on the adhesivity of the cell membranes. The model can be used to predict the effects of time variation of adhesions as a mechanistic basis for the dynamics of pattern formation. Once again, though, in addition to the calculations being based on an idealization and a caricature of cell shapes and interfacial tensions and free energies, such work treats pattern formation as a purely equilibrium process, determined by the initial conditions and energetic considerations — no self-organization is involved.

An alternative but similar approach to the study of surface tension has been proposed by Hoath [162], whose work is motivated specifically by the formation and topological properties of hair whorls. He argues that an analysis of skin tension is fundamental to problems of integumental pattern formation, as for instance the alignment of hair follicles is correlated with the local tension field (we are once again back to integumental patterning!). The important biochemical factors which have the required physicochemical features to be involved in surface tension are argued to be epidermal growth factor and similar binding molecules within the skin; surface tension is operationally defined as differential adsorption or adhesion of such molecular factors. For the purpose of this study, a finite element model applied to the relevant molecular and cellular mechanisms is proposed, although not developed.

Shaping of Epithelial Tubules A significant study of deformations in epithelial morphogenesis, with particular emphasis on the shaping of a tubular epithelium such as the hypodermis of an arthropod leg segment, was presented by Mittenthal and Mazo [240]. Epithelia are interesting in that they can exchange neighbours, making them fluid within the two-dimensional plane

[268]; with respect to motions out of the plane, however, they can act as elastic sheets. This study combines these insights, using both Steinberg's differential adhesion hypothesis and the assumption that epithelia behave as elastic sheets. Rearrangements of cells within blocks in the epithelium adjust the shape of the tube to that which minimizes the free energy, this being the difference between the energy of mechanical strain due to bending of the epithelium, and the work of adhesion among cells.

The paper presents an unusually unified approach; it gives a lengthy discussion of the mechanical properties ascribed to the epithelium and the influence of an adhesion field, and an analysis of processes that generate and regulate an adhesion field, all combined with biological implications that include the prediction of a scaling relation between the length and radius of a cylindrical segment, as well as extensive mathematical details using continuum representations of the fields and variational methods. Thus it is an excellent discussion of how patterns of strain and adhesion can provide the 'positional information' which directs deformation and hence regulates subsequent development. Again, this an equilibrium perspective, but it provides a good example of how the properties of epithelial sheets influence their structure.

5.3.2 Alternative Approaches to Epithelial Deformation

In the morphogenesis of organs, one of the significant problems is in studying the complex shape changes that take place in sheets of cells. As we have already noted, there are several distinct approaches to this problem. One fruitful line of attack, which we have treated above in section 5.2, is the reductionist paradigm: the identification of changes in cytoplasmic organization that correlate with changes in cell shape. Thus there is considerable experimental data on the involvement of the cytoskeletal microfilaments and microtubules in cellular elongation, motility and deformation, and some fairly detailed modelling has been done on the mechanochemical calcium/cytoplasm coupling. The approach we have just considered (section 5.3.1) treats the epithelium as a whole, taking a more global view of its deformations, but bases these on a specific local property, namely adhesion. We shall here briefly outline a variety of other studies of epithelial patterning, which largely have the character of descriptions and simulations, rather than explanations based on first principles.

Computer Simulations of Deformations As usual in the development of biological theory, computer simulations are required in view of the complexity of any available system equations. There are two basic approaches to the use of such simulations: Computer modelling may firstly be used to test the predictions of a theory; this approach is exemplified by almost all the work treated throughout this thesis (for example [175, 283]). The alternative is to attempt to *duplicate* the observed developmental changes in shape, by constructing a computer simulation. The role of individual parameters with biological interpretations can then be tested, in the hope of elucidating their significance in developmental shaping.

Such simulations are not directly connected to an understanding of mechanisms, as they aim merely to find *some* rules that reproduce the observed patterns, without initially asking whether those rules correspond to any actual underlying biological processes. If, however, more or less universal generative rules are 'discovered' by such simulations, they may be expected to have a counterpart in some universally applicable developmental mechanism, so that the

success of simulation attempts acts as an encouragement to search for appropriate genetic or epigenetic factors involved in the creation of the shapes under consideration [384]. In terms of our overall focus, such work is at best of peripheral interest to us, as self-organization is not directly involved in these studies — the patterns arising in the models, far from being novel, are frequently precisely those the simulation was designed to produce. Nevertheless, such simulations do show much potential for producing preliminary insights on what factors are likely to be most important for the generation of a particular structure; such indications can then be used in the search for a more mechanistic understanding.

Examples of Simulations An example of the work described above is a computer simulation of organogenesis, which reproduces the shaping of epithelial organs based on measurements of the primordium of the relevant organ [161]. This has allowed the identification of regions of morphogenetic activity and the shape changes occurring in those regions, that may not have been recognizable by inspection of the entire primordium. The intention was not to provide a theoretical interpretation of the motive forces involved, but to give a method of determining what kind of forces must be acting, and where, as an aid in designing experimental approaches.

Another recent finite element model has been suggested to imitate the morphogenesis of smoothly curved tubular epithelial rudiments [26]. The simulation of biologically realistic shapes by the use of a small number of generative rules corresponding to properties of the sheets (with due consideration also being given to the roles of local curvature, initial geometry and viscoelastic cell-cell linkages) is a promising indication that the observed shapes may be accounted for by the internal sheet properties and the requirements of mechanical equilibrium — that the shapes 'self-organize' in the sense that no external factors need to be invoked in the explanation.

Other Approaches A variety of other approaches is scattered in the literature. One example is an attempt to construct a phenomenological model which accounts in some way for the 'polarization' (apico-basal elongation) of epithelial cells [25]. Another approach is fluid-mechanical, considering the embryo deformations during gastrulation. In particular, a boundary integral formulation is used to calculate the distribution of tensions and surface moments during exogastrulation, by approximating the embryo as a viscous droplet surrounded by a thin membrane composed of cells which may exert passive as well as active forces [389]. Exogastrulation, which can be induced *in vitro* through chemical treatment of the embryo, is a situation in which the same deformations as in normal gastrulation are observed, except that the elongation is *outward*. Clearly, such deformations cannot be due to the active participation of internal organelles such as through filopodial pulling (as originally inferred from sea urchin experiments — see section 2.2.1), indicating that the mechanisms underlying normal gastrulation may also probably be ascribed to macroscopic surface force distributions and active cell repacking rather than the classically assumed filopodial pulling mechanism (see also [145]). Thus such a simulation, here based on an underlying, idealized, physical model, is able to aid in the discrimination between mechanisms, providing an alternative and complementary approach to the elucidation of the factors responsible for the shape changes and self-organization in development.

5.3.3 Geometrical Aspects of Surface Morphogenesis

The studies considered so far have considered *specific* physical mechanisms, predominantly adhesion and the associated surface tension, as driving forces for epithelial deformation, and have modelled the resultant shape changes. There have been several more general treatments of geometrical aspects of surface deformations, which have considered the response of sheets to unspecified given forces, the origin of which is not questioned.

The Generation of 'Real Form' An early study along these lines was made by Gierer (see [112]), who used shell theory, based on the assumption that the thickness of a cell sheet is considerably smaller than its radius of curvature, to simulate the generation of what he called 'real form' — that is, the morphogenetic shape changes and deformations, including the distribution of curvatures, that follow the laying down of a prepattern in the sense of the Gierer-Meinhardt theories [114]. He assumed that the steady state curvatures were describable by the minimum of some generalized potential, and by postulating a nonlinear form for this potential in terms of the surface areas, was able to simulate some processes of invagination and evagination, as well as the generation of elongated structures of various types. In contrast to the adhesion-motivated work presented above, this study did not consider any explicit physical interpretation for the proposed potential.

Cummings — Pattern-Surface Models Cummings has presented a number of rather abstract studies of pattern formation on cell surfaces. One of these models consists of two coupled second-order equations, one describing a pattern function on a surface, and the other an equation for the surface geometry which is affected by the pattern function and changes with time [71]. The motivation of the work is to present a 'unified treatment' of the processes of regeneration and duplication, gastrulation and segmentation; the model predicts a feedback between the pattern function and the surface shape, each influencing the other, with a Helmholtz-type averaging algorithm determining the generation of the surface geometry. An explicit identification of the pattern function is not made, although an interpretation in terms of adhesion molecules is proposed, with a variational approach considering the adhesive potential energy. Some applications are presented (and in particular, the point is noted that the ubiquitous formation of cylindrical tubes in development may be ascribed to their flat metric and hence energy-minimizing properties). On the whole, however, the work does seem somewhat removed from experimental application.

A later model, for the morphogenetic movement of surfaces composed of cellular monolayers, appears similarly abstract [72]. In this case, an otherwise unspecified 'morphogen' function m is introduced, and its defining equation coupled by a feedback relation to an equation for the surface, specified by its metric tensor g in a differential geometry formalism. The complexity of the equations precludes much analytical insight, however, except that regulation, or size invariance, is explicitly incorporated into the model; and only the simple case of axial symmetry has been studied, with a proposed application to gastrulation. Straightforward biological interpretations of the parameters and the 'morphogen', and the interactions that have been proposed for them, seem difficult. We have already seen a range of models that have generated many observed shapes of surface deformations on the basis of biologically reasonable assumptions, so that the need for such an abstract treatment is questionable.

A General Formalism for Surface Deformations The final treatment of surface deformations considered here does not aim to 'explain' any aspects of morphogenesis, but rather to provide a general formalism within which the deformation of a sheet in the presence of an isotropic local body stress may be treated. Hart and Trainor [157] point out that the problem of epithelial morphogenesis may be considered in two interrelated parts:

1. How do the forces, or differential growth rates, become distributed over the surface?
2. What is the resulting change in surface shape given some distribution of forces?

The first problem is that of pattern formation, which forms the predominant focus of this thesis, and may be due in particular to self-organizing mechanisms. The second is the conceptually 'simpler' situation of relating shape changes in a sheet to forces. Here there is no conceptual difficulty, no need for symmetry-breaking or other counterintuitive concepts, as the process is driven by the requirements of mechanical equilibrium and energy minimization; but it is of course the 'working out' of the pattern to produce 'real form' [112].

To the end of establishing a formalism for the effects of generalized forces (although only stretching forces, tangential to the surface, are considered), Hart and Trainor [157] combine shell theory in elasticity and the differential geometry of surfaces; under certain conditions the equations may be solved to give the surface metric tensor as a function of the local tension. Thus the intention of this work is purely geometrical, with no aim of providing a mechanism for the establishment or distribution of any of the forces considered.

5.3.4 Fields in Morphogenesis

In the last two chapters 4 and 5, we have considered a range of models, largely embodying self-organizing behaviour (although some other approaches were also described), for the continuous dynamics of developmental processes, which are more or less conducive to experimental verification. As exemplified by the somewhat extreme case of the work of Cummings considered above [71, 72], the connection with testable biological predictions, which would be expected to be a guiding force and hallmark of a useful model, is fairly tenuous in some of the work. This feature is frequently (and unfortunately) a sign of a more physics-motivated approach, which we have already seen in the discussions of self-organization: In physics a theory is often considered useful if it provides a new abstract explanatory *concept* or *paradigm*, in contrast to the far more practical, experimentally-oriented biological approach.

We now proceed to the discussion of two concepts which are in some sense almost totally abstract, although with proposed applications. The categorization of these with in the framework of this thesis is somewhat unclear; the concept of a field has been retained in this chapter for its putative connections with developmental problems such as surface patterning, cleavage, and regeneration, while the discussion of catastrophe theory has been relegated to the next chapter, being essentially in a 'class of its own'.

The Field Concept

The concept of a **morphogenetic field** has been around in embryology for decades, but it has always tended to be somewhat hazy and ill-defined, in the sense of being an 'explanatory' term

when no actual explanation was available. As Slack points out [327], the term 'field' has two clearly distinguishable embryological meanings, which he labels the 'physical' and 'agricultural' forms. With the latter, which is the commonly accepted usage and which we have already encountered, there is little quarrel: an embryonic field simply denotes that region within which a certain process, such as an inductive interaction, occurs.

Thus a limb field, for instance, is that part of the mesodermal mantle of the embryo in which, around the time of gastrulation and subsequent to it, interactions occur which will eventually under normal developmental conditions result in the formation of a determined limb rudiment and ultimately a limb. Similarly a given process, such as differentiation to a certain cell type, occurs within a domain or 'field' of the embryo. The exact nature and location of this field depends strongly on the process involved, however, and can be specified and delimited accurately only in normal development. For an inductive interaction, for example, neither the cells which are part of the field, nor the spatial boundaries of the field, are fixed and well-defined, as is demonstrated in situations where the cells being induced, and the inducing region, are transplanted, respectively. Thus the concept of field in this sense is more descriptive than normative, and is thus not essential as an explanatory construct.

The term 'field' was also, however, widely used as an undefined 'explanation', whereby a process that was not understood was given a name to 'hide ignorance'. We have already encountered a common case of this, the historical usage (prior to the work of Wolpert [375] and Crick [67]) of the concept of a 'gradient field' as a pseudoexplanation for a varying property or a signal whose nature was a mystery. In response to such abuse, and in the light of the confusion of meanings — we shall meet the physical interpretation shortly — the term 'field' has largely fallen into disfavour. In the cases where such an 'agricultural' concept is still useful, other terms have been introduced to denote an area of tissue within which a process occurs. One such term is 'polyclone', which refers to a group of cells, generally descended from a common progenitor, which make up a developmental compartment (arising from *Drosophila* studies — see section 2.2.3). The other is 'equivalence group', used, most usually in research on the nematode *Caenorhabditis*, to describe a group of cells with a common competence. Neither of these terms appears to have caught on much outside their respective organism-based communities [327].

Fields in Physics The alternative usage of the field concept is that common, and indeed indispensable, in physics (see [352]). The fundamental fields, which mediate the basic forces of gravitation, electromagnetism, and the strong and weak nuclear forces, are abstract mathematical entities in themselves, which obey dynamical equations even in a vacuum, and manifest a field energy either alone or in the presence of matter. Their influences are ubiquitous, in spite of their invisibility and mysterious, not fully understood nature, and it appears that the formulation of the fundamental physical theories of the universe may be impossible without fields (although sophisticated attempts at action-at-a-distance formulations have been made). In physics, the field concept has certainly proved its worth.

But the term 'field' in physics is used more broadly than solely in reference to these fundamental fields. In the more general usage, a field is *a space-time function obeying some dynamics and describing some property of the system.* Thus in physics there are for instance velocity fields in hydrodynamics, and pressure fields in acoustics. It is this more general definition which may also be employed in embryology. Thus it is reasonable to speak, in particular, of the con-

centration fields of morphogens, the key biochemical species which are purported to control developmental patterning; similarly, one can refer to the viscoelastic strain fields present in the cytogel, and stress or deformation fields in the epithelium. In this sense, virtually all the work we have considered so far may fall under a 'field' description, with field equations providing the quantitative dynamics of the field variables. The concept of a field is thus a natural one here, fully compatible with everything we have seen so far, and thus also not particularly novel or helpful — we can just as well do without it.

Field Descriptions of Morphogenesis Some workers, largely in an attempt to place developmental biology on a similarly general and powerful theoretical and mathematical foundation to physics, have postulated generalized developmental fields to account for some given morphogenetic situation, without necessarily any actual, or hypothesized, biological interpretation for the field variables. Thus Brandts and Trainor, for example, explicitly state their "ambition of bringing about a unification of the formalism, phenomenology and concepts of physical theory with the foundations of theory in biology" [36, p.56], and to this end introduce a morphogenetic field as an ordering influence for pattern formation and a vehicle for positional information, as we shall see below. Such a quest is justified by the qualitative similarities exhibited between biological and physical fields, such as the 'smoothness' property of positional information, which corresponds mathematically to differentiability of the field variable.

An interesting point brought up by these authors [36] is that dynamical laws in physical fields (and hence by the assumed extension also in biological fields) may have alternative, equivalent mathematical representations with different interpretations. For example in particle motion in classical dynamics, there is a differential equation formulation in terms of the Euler-Lagrange equations, which expresses local field values and interactions; and there is the Hamilton principle of least action over any segment of the path, which expresses the dynamics in terms of global properties. The implication for biological fields is that *global* order and organization may thus be thought of as due to *local* influences, without having to assign any non-physical 'action at a distance'; this is indeed the basic lesson of self-organization.

Field Descriptions of Cleavage

Catalano and Eilbeck The concept of a general field description of embryonic processes has been used by two sets of workers for the study of the *cleavage* process, that is, early embryonic cell division. Catalano and Eilbeck [52] postulated a surface 'cleavage field', which may be interpreted as the surface density of sources of active transport of ions, or of specific binding sites or functional membrane receptors; the detailed meaning is left open, outside of the more abstract field model. This two-dimensional scalar field associated with the membrane of the egg was assumed to satisfy a Laplace equation, and the geometry of the cleavage planes was accordingly associated with the geometry of the level surfaces of ionic concentration (for the first interpretation of the field variable). The modification of the surface field during cleavage was also considered, and a simulation performed on a two-dimensional simplified model, which displayed many interesting patterns of cleavage, similar in many ways to those observed on three-dimensional embryos.

Comparisons of the two-dimensional simulations with experimental observations both of

normal cleavage and the behaviour in response to different treatments, such as compression, centrifugation and action by chemical agents, were made and seemed to reveal no obvious discrepancies; the true experimental tests would, however, clearly be in three dimensions. In spite of the experimental comparisons, and attempts to propose a realistic molecular interpretation for the field variable, the theory ultimately stood independently as an abstract entity designed merely to simulate patterns of cleavage on the assumption of some unspecified field.

Goodwin and Trainor Such abstractness was the case also for another description of cleavage published shortly after the above, by Goodwin and Trainor [130], where speculations about the nature of the descriptive field concluded the paper almost as an afterthought — one possibility presented was the assembly and orientation of microfilaments in terms of some relevant order-disorder parameter. The field description here arose out of a comparison of the typical cleavage lines of holoblastic eggs (those which cleave totally, with radially symmetric cleavage) with the nodal lines of selected harmonic functions on the sphere.

The field variable was assumed to satisfy a minimization principle for a generalized surface energy function, which generated spherical harmonics as solutions, with an associated surface energy. The cleavage process was then considered to occur along a unique succession of planes, obtained by a set of selection rules to choose an appropriate sequence of spherical harmonics, and in this way the typically obtained first few cleavage divisions were simulated. Modifications of the usual pattern, such as unequal cleavage and bilateral rather than radial symmetry, were accounted for by simple modifications such as scale changes along the coordinate axes. In this way a series of rules which generates typical cleavage patterns was obtained, so that an explanation for cleavage 'in principle' was found (pending, of course, suitable interpretations of the field variable and selection rules).

Although especially in this last description [130] no serious search for biological correspondence with the field assumptions was made, the fact that the observed patterns could be imitated by the choice of an appropriate set of rules was considered a strong, almost sufficient, indication of the spontaneous expression of spatial ordering principles inherent in organisms, described and interpreted as fields. The fields were thought to be intrinsic properties describing the state of the whole organism, and undergoing systematic transformations conforming to constraints defined by selection rules, with the entire process being broadly self-regulating. This philosophical approach, looking for a conceptual, theoretical or physical basis to account for phenomena, underlies much of the work of Goodwin and Trainor, even that in which a detailed model is based on cell properties and successively refined to approach experimental verification (for example [131] — see section 5.2.2).

Abstract Field Approaches — Goodwin and Trainor

Goodwin's Structuralist Philosophy We have already considered the polar coordinate model of French *et al.* [105] (see section 4.2.6), in which a set of positional coordinates is postulated in conjunction with a class of rules, and numerous predictions arising from these rules are found to be in excellent agreement with experiments, especially for epimorphic regulation. A feature of this work is that the rules are expressed not in terms of genetic or molecular properties, but are of a purely *relational* nature. Goodwin [126] regards this as a highly significant

characteristic, arguing that the problem of biological self-organization must be clearly described in terms of an abstract reduction to laws and rules, deliberately avoiding any *a priori* material reduction to underlying mechanisms which are nowhere fully understood anyway. He seeks overall structuralist principles and global organizational levels and constraints, contending that it is such constraints on a holistic organismal level which form the ultimate biological description; the genes and gene products merely act within an organized context.

Thus Goodwin strongly opposes any reductionism of development to a 'genetic program', as he claims that in the absence of higher-level laws and principles, the morphogenetically generated forms are rendered epistemologically irreducibly complex. In his view, the principles of morphogenesis consist of common organizational constraints, in the form of fields — these define biological universals — together with specific constraints characteristic of individual species, which may be genetically specified. The proper field description, he feels, is embodied in the spatial averaging or intercalation rule applied to field values, as exemplified by the polar coordinate model. The mathematical equivalent of this spatial averaging rule is then the most general field equation in physics, namely Laplace's equation, which was already applied in the cleavage model discussed above [130].

Goodwin's radically holistic, structuralist view, as formulated in [126], seeking global organizational principles in contrast to, and almost in rejection of, a local molecular or genetic understanding, is extreme, but serves as a useful antidote to the diametrically opposed view, a rabid reductionism ignoring any overall dynamical principles. We will however see in the next chapter the demise, in terms of impact on developmental thinking, of a similarly extreme and global philosophical view, that of catastrophe theory. For alternative, more biologically orientated and correspondingly less extreme formulations of Goodwin's views, see [125, 127].

Trainor — Fields in Regulation and Regeneration A similar emphasis on the usefulness and conceptual primacy of fields has been made by Trainor and his students (see [352]). He also notes that, although it was not initially labelled as such, the polar coordinate model [105] is a field model, assigning azimuthal field values for the developing system of interest, such as the regenerating limb. Trainor and coworkers have sought to give a mathematical description to this model and extend it to the cases where the predictions of the original polar coordinate model [46, 105] are in conflict with experiment.

Thus Totafurno and Trainor [351] posited a developmental field to account for the results of epimorphic regeneration and transplant phenomena in amphibian limbs, and in particular to account for the phenomenology of supernumerary production. A number of plausible guidelines, regarding smoothness properties, nonlinearities, normalization and simplicity, were followed in postulating the equations for the vector field, the magnitude of which was then taken as a measure of 'distalness'. Solutions of the equations revealed bifurcating behaviour and field patterns in essential agreement, given appropriate interpretations, with the polar coordinate model and with results of grafting experiments. The question of the nature and interpretation of the developmental field was, however, deliberately avoided, as the model was directed at a higher level of description. Although self-organizing behaviour and breaking of symmetry are displayed in this model (having been explicitly incorporated into the equation formulation), in the absence of a biological correspondence with the model parameters, this field description cannot be taken as a demonstration or instance of biological self-organization.

A similar nonlinear field approach was taken in the study of intercalation in morphallactic regulation (where regulation occurs without growth at the cut surface; rather, there is dedifferentiation of cells and rearrangement of positional values) by Brandts and Trainor [36]. A generalized morphogenetic field was again postulated, and assumed to satisfy a condition of the minimization of a nonlinear energy functional, with certain additional smoothness conditions — these defined its dynamic behaviour, based on biologically plausible requirements. Here, as in [351], the equations were chosen to be as simple as possible within the requirements. The model was solved numerically, and two main classes of solutions, with normal and reversed symmetry, were discovered, with transitions occurring between them as the system size is altered; these solutions were taken to suggest that in morphallaxis, unlike in epimorphosis, reverse intercalation can occur even without the presence of a discontinuity.

These solutions were given a detailed biological interpretation in the regulation of *Tetrahymena*, a ciliated protozoan [37]. *Tetrahymena* doublets are arrangements of two cells, fused side by side, which initially have, for example, two complete oral structures. During the regulation of these doublets, a configuration with three oral structures occasionally appears, before the subsequent conversion to a singlet with one oral structure. The proposed field model was able to account for this surprising behaviour in a natural way, and make detailed predictions about the location and orientation of pattern elements such as the oral structures. The success of this application was claimed to vindicate the application of the physics-oriented field concept to biological pattern formation, and more generally to lend support to the unification of physical and biological theory. In contrast to these somewhat grandiose claims, apparently based on very few successful and novel applications, it appears however that while it is admittedly some success to be able to account in some way for an otherwise unexpected and counterintuitive experimental observation, it is not nearly the case that field theory is now established in biology.

Assessment of Field Approaches The highly abstract usage of the field concept championed by Goodwin and Trainor runs counter to the trend of most biologists to stick to experimental observables and not to dabble in such abstract concepts; and indeed it must be argued that while a concept that in some way accounts for otherwise mysterious observations must at least be taken seriously, only in the face of a biological interpretation and potential for experimental verification or falsification can a model somehow be accepted. Biological theory is not nearly yet at the stage where more general and abstract *reformulations* of the major concepts are feasible and useful, for we do not yet know of *one* model or concept that is indisputable, accepted beyond reasonable doubt.

On the other hand, extensive experience in physics has taught us the usefulness, indeed the indispensability of *paradigms*, of *conceptual modes of thought* about a problem, and throughout this thesis the self-organization paradigm has indeed demonstrated its worth as an explanatory and descriptive construct in a wide variety of developmental situations and models. Thus the introduction and advocacy of a physics-like field concept can but be beneficial in broadening the range of ideas from which, ultimately, a 'unified theory of development' will hopefully one day be constructed. At present, however, such abstract field theories, though conceptually attractive in their simplicity and breadth, are not saying all that much that is new or interesting, except in terms so abstract that the connection with biology is tenuous or difficult to find; and the self-organization inherent in such models is not novel, having been incorporated by the choice of the model equations. Thus we essentially need to reserve judgment on field descriptions.

In the latter parts of this chapter, we have tended more and more towards abstract descriptions of development, with the link to experiment and to biological reality becoming ever fainter. Except possibly in the final field approaches, there has nevertheless been the common feature of cells participating in their own development, as opposed to the slaved response to some previously laid down prepattern or positional information system. We now proceed to consider alternative approaches to the description of development which appear to have tended in some cases to eschew strict biological realism, beginning with possibly the most abstract and, by now, most controversial and discredited of all approaches, that of catastrophe theory. We then continue with an overview of discrete models, which form an instructive and useful counterpart to the approaches on which we have so far focussed, of continuous dynamical systems. These models do not purport nearly to complete a catalogue of mathematical or theoretical approaches to development — for example, entire bodies of theory on informational and computational aspects have been omitted or skimmed over — but they are included as they constitute the major remaining approaches that are, in some way, connected with self-organization in biological development, but form neither continuous prepattern nor cell-participation models.

Chapter 6

Two Alternative Approaches: Catastrophe Theory and Discrete Models

The field approaches have introduced a somewhat philosophical, conceptual element into developmental theorizing, arguing by direct analogy with physics and attempting to place developmental biology on a similar firm mathematical and conceptual foundation. The diverse more concrete models for pattern formation and morphogenesis we have encountered in chapters 4 and 5 have also taken the application of mathematics to physics and chemistry as a prototype, assuming functional forms for the interactions considered most important and deriving quantitative differential equation formulations for the models, in a comparable manner to the tried and tested, and highly successful, procedure in physics and chemistry. Such a physics-led approach has undoubtedly contributed much to our understanding of development, as the success of the above models in the conceptual understanding of otherwise mysterious self-organizing processes has shown.

The unique status of biology, fundamentally distinct from physics, must however also be taken into consideration; by virtue of their functionality, their emergent properties arising from hierarchical and interacting levels of organization, biological systems acquire that characteristic and irreducible feature we call Life. Thus some have argued that a *new* mathematical approach adapted specifically to biology (and possibly other 'soft' sciences) is needed, as opposed to ill-fitting paradigms borrowed uncritically from other 'hard' sciences for which they are better suited. This has led to at least two major approaches: discrete systems have been studied, both for mathematical and computational convenience and simplicity, and in reflection of the fundamentally discrete cellular structure of organisms and combinatorial nature of the genetic code presumably underlying much, if not all, of development; discrete approaches will be discussed in section 6.2. The other approach, once much-heralded but today largely ignored, is that of catastrophe theory.

6.1 Catastrophe Theory

Our discussion of catastrophe theory will here be rather brief; as any attempt at a full discussion of the mathematical background and implications is well beyond the scope of this work, and the current impact on developmental biology theory appears minimal.

Catastrophe theory, and its applicability, may be interpreted broadly, from a more philosophical point of view, as a 'theory of general morphology' [387, p.633] or more narrowly, in terms of the theory of singularities of smooth functions (known also as 'elementary catastrophe theory') and applications of this theory. The two principal exponents of catastrophe theory, Thom and Zeeman, have taken quite different views on the relative importance of generalized *versus* elementary catastrophe theory [328], so we discuss both of these, beginning with elementary catastrophe theory, which constitutes the more mathematical approach.

6.1.1 Mathematical Aspects of Elementary Catastrophe Theory

The mathematics of the theory can only be described very briefly here; for further elementary introductions, see for example [4, 137, 309], and for fuller discussions, see the compedium of papers by Zeeman [387], or particularly the seminal book by Thom, [344]. In brief, catastrophe theory describes the behaviour of a dynamical system in terms of the stationary values of a function f (often called a potential function) associated with that system, and depending on state x and parameters μ. A 'catastrophe surface' representing these stationary values may be created by the loci of the maxima and minima of the potential function; trajectories of a state point on that surface represent transitions generated in the system by changes in the constraints, or control parameters, of the system. The problem of interest is to find standard (canonical) forms of these surfaces, or 'unfoldings' of f, for 'generic' f (where *genericity*, considered simply, refers to a 'typical' property, that is one holding for an open dense subset in a suitable function space). An aim is to classify the generic *singular phenomena*, called **catastrophes**, occurring within the appropriate mathematical context.

The singularity theory of smooth functions leads then to *elementary catastrophe theory*, in which it has been possible to classify the singularities of systems depending on few parameters; in particular, if f depends on at most four controlling parameters ($\mu \in \mathbf{R}^4$) seven canonical forms exist, representing all the possible transitions available to generic gradient systems depending on four parameters [137, 328, 382]. We have already considered in some depth the concept of bifurcations, and the notion of a sudden qualitative change in the equilibrium and stability behaviour of a system once the parameter(s) cross a bifurcation set (see appendix A.3). Catastrophe theory tells us that providing the dynamics are of a particular gradient form (represented generally by $\dot{x} = \nabla f$) with no particular symmetries, and the number of parameters determining the sudden change is not too great, the number of qualitatively different bifurcation sets (where two sets or equations are equivalent if one can be reduced to the other, or both to some standard 'normal form', by a suitable change in coordinates) is surprisingly small, namely seven for the case of up to four parameters (more generally, if $\mu \in \mathbf{R}^5$, that is there at most five relevant parameters, then there are eleven distinct elementary catastrophes and $x \in \mathbf{R}^2$, that is no more that two state variables need to be taken into account in determining the catastrophe behaviour) [309, 316].

The mathematical concept of *structural stability* is crucial to the foundations of catastrophe theory: Models and equations exhibiting this property are assured of retaining their qualitative behaviour under small perturbations of the system. In view of the perturbations constantly present, the value of a model in one of the applied sciences might reasonably depend on considerations of stability and genericity. This, in fact, motivated much of the development and application of catastrophe theory. In particular, Thom was motivated by the problems of biological development, as indicated by the title of his book, *Structural Stability and Morphogenesis* [344]. He was interested in global regularities in biology, and the apparently sudden and discontinuous changes observed during the generation of biological form. While the theory has had important ramifications in mathematics and physics (for example the study of caustics) — see [309] for a description of some of these — and other (speculative and controversial) applications to the life and social sciences (including to evolution, the structure of societies, and linguistics) exist, catastrophe theory was indeed created first with applications to developmental biology in mind, and these pervade large sections of Thom's book [344].

6.1.2 Applications to Developmental Biology

Thom: Generalized Applications

The applications of catastrophe theory to developmental biology that Thom proposed were directed largely at giving a mathematical expression to the conceptual ideas expressed by Waddington, regarding 'chreods' and the 'epigenetic landscape' (see for example [316, 382]). The idea of the *epigenetic landscape* was a phenomenological model, meant to provide a heuristic aid to the understanding of the undoubted stability of developmental processes: the developmental system of an organism was portrayed as a mountainous terrain, with the valleys representing possible pathways along which development (represented as the rolling motion of a ball down the landscape) could in principle take place. The ball was typically constrained to a particular valley even in the face of minor disturbances; only major perturbations could cause it the ball to be sufficiently deflected off its course to cross a watershed and enter another developmental pathway. This was an expression of the stability of developmental processes. In the dynamics on the epigenetic landscape, the valleys represent attracting trajectories, labelled 'chreods'.

Thom's goal was to give a more rigorous mathematical formulation of this heuristic picture of chreods, epigenetic landscapes and morphogenetic fields, for instance replacing the epigenetic landscape by the mathematically more well-defined catastrophe surface. He discusses [344] several applications to early morphogenetic movements and form changes in the embryology of amphibians and birds, mainly by using vague analogies between catastrophies and the local (but averaged, smoothed) geometries and discontinuities observed in morphogenesis. 'Models' for gastrulation, the primitive streak in birds, neurulation, the development of the vertebrate limb and other systems are presented, in topological terms, by linking the observed changes to one or other of the elementary catastrophies.

The motivation he gives is that a fundamental problem in biology is to account for the integration and coordination of local processes and mechanisms to form a coherent and stable global structure, a fact we have observed throughout this thesis; his point of view is that "the fundamental problem in biology is a topological one, for topology is precisely the mathematical discipline dealing with the passage from the local to the global" [344, p.151]. Coming from such

a perspective, which essentially and intentionally ignores the extensive body of experimental data, it is almost inevitable that Thom's models are too abstract to be at all useful, and seem to provide mainly a theoretical language within which discontinuities may be described, without providing any new insights or experimentally testable predictions. As we shall see later, Thom seems never to have intended to set up a testable, or falsifiable, theory.

Zeeman: More Specific Applications

More specific applications to developmental biology have been proposed, primarily by Zeeman, whose interest has been to establish detailed applications of (elementary) catastrophe theory to a variety of sciences. In view of the controversy surrounding them [385] and hence their somewhat dubious status, these applications will not be discussed in any depth. The cusp catastrophe, depending on two parameters and thus after the fold the simplest catastrophe, features prominently and regularly in such applications, and it is usual that a cusp catastrophe diagram is drawn on the basis of a few elementary assumptions on the continuity and genericity of the model required, and the premise that the simplest catastrophe fitting the criteria must be the most likely; and then 'predictions' are derived and interpreted into biological conclusions.

Waves in Embryology The major 'application' consists of Zeeman's studies of how homogeneous tissue of an embryo differentiates into two types separated by a frontier [386] (assuming thus that the two differentiated types of cells are well separated). Without considering how the homogeneity might be broken, a question which has concerned us throughout much of this thesis, Zeeman proposes that the behaviour subsequent to differentiation tends to follow a certain pattern, namely that the frontier between two cell types always forms to one side of its final position, and then moves through the tissue before stabilizing. He reaches this conclusion by translating the assumptions of 'homeostasis, continuity, differentiation and repeatability' into mathematical terms, and using 'Thom's theorem' (apparently due to Whitney [345]) of the classification of the elementary catastrophes to 'derive' a cusp catastrophe as the most probable. This leads to certain quantitative predictions, including an estimate of the initial velocity of the frontier wave. The wave thus formed is called *primary*, that is, its mechanism of formation is space and time dependent. Zeeman suggests that this wave, which is often a hidden wave of cell determination, is then followed by a *secondary* wave of cellular activity, a visible manifestation of the changes that have occurred, which is a purely kinematic wave, a series of local events occurring at a fixed time delay after the passage of the primary wave.

Clock and Wavefront Model for Somitogenesis Applications of these concepts of primary and secondary waves were presented to gastrulation and neurulation in amphibia, and to the culmination of cellular slime moulds [386]. The most well-developed embryological application is however one that we have already met (see section 4.2.5), namely the clock and wavefront model for somitogenesis, proposed together with Cooke [64]. Here the primary wave corresponds to the gradient we considered in the earlier discussion; it can be any means of creating a graded distribution of positional information. The cells are also assumed to possess intracellular oscillators, and the phase-linked interactions between these clocks and the positional information cause a wavefront of rapid cellular change, a 'secondary wave' of changes in adhesive or locomotory properties, to propagate in an anterior-posterior direction in a succession of separate

populations, causing the segregation of somites.

We have already discussed this model and noted that it has not been formulated rigorously enough to provide testable or falsifiable predictions (section 4.2.5); and we have met another, traction-based model that appears to provide a plausible explanation for somitogenesis [15] (section 5.1.1). Of interest now is just that the consequences of combining the clock and gradient were suggested by catastrophe-theoretic considerations (see [388]), based on the prediction of primary and secondary waves; secondary waves are required in view of the fact that experimental observations show somite formation to be unimpeded by cuts in the tissue ahead of the wave of somite formation. There does not appear to be anything in formulation of the theory, hypotheses or results that requires catastrophe theory — we were able to discuss it in chapter 4 before encountering catastrophes — as noted also by Cooke: "I, at least, do not regard any of the predictions of the model in which I am involved as being deeply distinctive to catastrophe theory" [385, p.762].

Catastrophe-theoretic Applications: Initial Optimism

Other applications of catastrophe theory to developmental biology are in a similar vein; they do not necessarily yield concrete predictions, or alternatively, are not dependent on catastrophe theory for their results. We have already indicated some problems with the models in our discussions so far. Nevertheless, all this was not apparent in the early years of catastrophe theory, in the first half of the 1970s, and there were extremely high hopes that catastrophe theory provided a new and powerful way of dealing with discontinuous phenomena, and hence a potential method for describing the evolution of forms in all aspects of nature, and a profound insight into the world, the mathematical description of which had since Newton been trapped in the 'straightjacket of continuity'. Indeed, there was tremendous public interest in catastrophe theory, and its vigorous proponents proclaimed a 'revolution' in mathematics: "Properly understood and exploited, this ever-expanding web of concepts promises mankind a unique weapon against ignorance and a profound insight into the universe" [333].

6.1.3 Critique of Catastrophe-theoretic Applications

The Controversy

The bubble of enthusiasm burst in about 1977, when serious doubts began to be raised about the usefulness and accomplishments of catastrophe theory, especially in the biological and social sciences. A great deal of controversy was created, with sharp public statements and attacks being made by protagonists and antagonists of the applications of the theory. It became clear that public relations and hype had played a not insignificant role in the astounding success of catastrophe theory, and that the applications tended to be based on somewhat weak assumptions and analogies, on easy-to-understand pictures of a cusp catastrophe together with intimidating references to the 'deep' classification theorem of Thom. The promise of profound applications based on abstruse mathematical arguments few could understand impressed many, and it took serious and public challenges to dispel the misconceptions.

Doubts about the applicability of catastrophe theory already began to be raised early (for example [136]), but were intensified, with Smale, himself a leader in the study of dynami-

cal systems, feeling that catastrophe theory "has limited substance, great pretension and that catastrophe theorists have created a false picture in the mathematical community and the public as to the power of [catastrophe theory] to solve problems in the social and natural sciences" [328, p.1360–1]. But the sharpest, most virulent attack was due to Zahler and Sussman, who criticized the applications of catastrophe theory as being characterized by "incorrect reasoning, far-fetched assumptions, erroneous consequences, and exaggerated claims" [385, p.759]. In their *Nature* article, which stirred up much controversy, the authors were highly critical of arbitrary, vague, misleading or trivial uses of both mathematics and experiment; the detailed criticisms were contained in a longer paper in *Synthese* [340].

The heated controversy aroused particularly by these public attacks on catastrophe theory died down within a few years, and the 'fad' passed, enabling a more rational assessment of the value of this approach in general, and to development in particular, to be made. Apart from isolated more recent attempts motivated by catastrophe theory (for example [315, 316]), applications to developmental biology appear to have faded away. It will be useful to attempt to pinpoint some of the reasons for this.

Weaknesses of Applied Catastrophe Theory

As we have already noted, the 'applications', for example the clock and wavefront model, have frequently had fairly little to do with catastrophe theory in the formulation of the model. They have also depended on vague appeals to concepts such as 'genericity', important in the mathematics, but with a doubtful biological interpretation. For example, Zeeman's work on primary and secondary waves in developmental biology [386], in which he 'shows' that a frontier between differentiating cells must move, is caricatured by Zahler and Sussman: "...all his theorem says is that if nothing exceptional happens, then the frontier moves. Zeeman's 'proof' consists of no more than the observation that, if the frontier did not move, that would be quite exceptional" [385, p.761]. The models of Thom are even less specific and well developed than those of Zeeman, which of course has enabled them to cover a wide range of topics, but with a certain superficiality [328]. The failure of catastrophe theory in developmental biology is not solely due to its weak use or misuse, however, but also the result of weaknesses with regard to potential applicability in the mathematical theory itself.

Mathematical Inappropriateness Thom's theory of structural stability refers first and foremost to systems with a finite number of degrees of freedom, described by ordinary differential equations. The classification of such systems, and more particularly potential systems, leads to elementary catastrophe theory, which has largely been used in the applications to morphogenesis. This classification depends, however, on certain restrictive mathematical conditions, which exclude, for example, Hopf bifurcations leading to limit cycles, as well as the possibility of any continuous dependence on space and time which biological structures are presumed to have: partial differential equations with infinite numbers of degrees of freedom are excluded by catastrophe theory, which can take into account only a parametric dependence of, for example, local reaction rates, on spatial position. It was early recognized [275] that this limitation was serious, and excluded most biological systems of interest. In particular, the spatial symmetry has to be broken *externally* of the theory, by assuming an appropriate spatial dependence of the parameters, in order to create spatial structure, and thus catastrophe theory gives no explana-

tion of *de novo* pattern formation, only of its transformation to more complex forms. This is in contrast to the concepts of self-organization and symmetry-breaking which we have considered at some length, which deal with the *origin* of structure and pattern.

It is clear that discontinuities and singular phenomena occur in development, at any rate in a mathematical idealization of properties such as chemical concentrations which on a detailed scale are continuous. Thus the goals of catastrophe theory, to study singular phenomena in a wide variety of contexts, appear reasonable for development. The trouble is that the program of classifying the catastrophes, or generic singularities, occurring within a particular context has been carried out for *smooth maps* — leading to elementary catastrophe theory — but in few other contexts. In particular, it appears that a reasonable multi-parameter bifurcation theory for vector fields can not be constructed; dynamical systems and vector fields are much 'messier' than functions [137]. Catastrophes in other contexts are referred to by Thom as 'generalized catastrophes', and frequently appear to be invoked when the elementary catastrophes cannot account for some phenomenon (for example, the formation of symmetric feather rudiments on a chicken embryo is referred to as an example of a generalized catastrophe [344, fig.21]) but they are not properly developed. It now appears as if a systematic theory is not in general possible [137].

The symmetries in feather rudiments alert us to another point: functions with certain symmetries are nongeneric in the space of all smooth functions, and are thus excluded in the general theory; if however a special symmetry constraint, possibly experimentally dictated, is placed on the system, the observed catastrophes are different from those described by elementary catastrophe theory. It all depends on the 'mathematical universe', the context within which one is working [137]. As physical and biological structures do frequently display nongeneric symmetries, elementary catastrophe theory does not suffice to treat such cases. Hence in a general context, and more specifically as regards potential applications to developmental biology, elementary catastrophe theory (the only aspect which is mathematically fairly well developed) can describe a *subset* of possible behaviours, but not nearly all, and must indeed give way to a more general dynamical systems approach, and to partial differential equations such as reaction-diffusion equations, when it comes to useful applications.

Catastrophe Theory as a Philosophical Approach

We have already noted the differing viewpoints of Zeeman, whose interests lay mainly in the application of elementary catastrophe theory, and Thom with respect to the usefulness and manner of applying catastrophe theory. Thom recognized early that the unjustified hopes that were raised about the potential applicability of the theory, were due to some extent to an "overoptimistic (and not always enlightened) vulgarization" [346, p.32], and that it was hence inevitable that the initial euphoria would be replaced by criticism of the pragmatic inadequacy of catastrophe-theoretic models, criticisms which were largely well-founded [347]. His point of view was that the *practical* usefulness of catastrophe theory was very dubious, except in some cases of physics, for example, where rigorous applications could provide precise quantitative predictions. For biology and the social sciences, however, the usefulness lay in *qualitative* models, which are essentially hermeneutic or interpretive; they provide a schema for thinking about phenomena, and enable analogies between different spheres of experience to be created. He calls this 'soft theorizing' [346]; where only qualitative understanding may be sought, on a local scale.

Thom rejects the idea that scientific models must necessarily lead to quantitative prediction; for him the value of catastrophe theory is as a classification of analogous situations, as placing the heuristically important notion of *analogy* on a formally mathematical, geometrical basis, as an extension of purely verbal arguments. Such geometrization, he feels, promotes a global view, and a new manner of thinking about phenomena from a range of fields ranging from biology through sociology to linguistics and semantics. Thom sees in catastrophe theory (both in its 'elementary' and 'generalized' manifestations) the potential for a general theory of forms, for an understanding of morphologies and their creation — for, given any observed morphology, one tries to construct a corresponding catastrophe theory; if this construction succeeds, the model is appropriate and the form falls into the general schema, while if unexpected features arise that cause the catastrophe construction to fail, this indicates that the morphology is interesting and deserves further study and the construction of a more complex model. On such an abstract, qualitative basis, as a 'theory' of forms and analogies, Thom feels that the conceptual, or epistemological, interest of catastrophe theory is beyond any doubt [346] (for a more detailed discussion, see [347, chapters 6–7]).

Conclusion: the Legacy of Catastrophe Theory

Such considerations may well be of philosophical interest — it is not appropriate here to attempt a detailed evaluation — but there appears little of *practical* use, other than vague and speculative analogies, for studies of pattern formation and morphogenesis in developmental biology: an assessment with which Thom would no doubt concur. So now that the excitement and controversy has died down, what has catastrophe theory left us?

Clearly, there have been exaggerated claims and unrealized hopes. Nevertheless, catastrophe theory may provide a basis for evaluating models; if, for example, the geometric observations are not consistent with elementary catastrophes, then models based on generic families of smooth functions, which would lead to such catastrophes, are not feasible. This gives a criterion for *eliminating* models, but helps little in the *construction* of models or the assessment of their *correctness*. The most enduring benefits of catastrophe theory, apart from its undoubted mathematical interest and profundity, are probably the renewed emphases on nonlinearities and discontinuities, and the interest in the philosophical ideas of structural stability and genericity, which were stimulated, and which have changed the way certain problems have been approached. Lastly, the catastrophe theory 'débâcle' has led science to be more careful of 'revolutionary' theories, to assess them more critically and thus to maintain a more balanced perspective on the value of novel concepts and approaches. Such an improved attitude is in itself a lasting and valuable legacy of catastrophe theory. Specifically to developmental biology, however, the bequests of catastrophe theory appear minimal.

6.2 Discrete Dynamical Systems and Iterative Rules

Our analyses so far of developmental mechanisms and self-organization have focussed on the study of *continuous* dynamical systems and their properties. This is in concordance with the traditional approach used in physics, in which realistic models based on underlying physical laws usually result in systems of (nonlinear) integro- and partial differential equations. This 'bottom-

up' approach to modelling has many advantages, such as permitting a quantitative matching of experimental results to the predictions of a model derived from the presumed mechanism, and a consequent detailed understanding of the processes at work in the situation under study. Furthermore, the powerful analytic tools developed for the study of continuous systems and differential equations are available for the analysis of the mathematically formulated model. We have already experienced some of the power and benefits of this approach through the understanding of self-organization obtained by the study of reaction-diffusion equations, with the aid of theorems for the existence and uniqueness of solutions, bifurcation and asymptotic techniques to gain a feel for the solution behaviour, and so on (see appendix A).

Motivation for a Discrete Approach to Modelling

But the 'natural' differential equation description is not the only and not necessarily the best one, and indeed entails a number of theoretical and practical difficulties [97, 371, 372]. Firstly, in physics a theoretical approach derived from first principles and fundamental laws is feasible and valid since a mathematical formulation and understanding of such laws exists in general, and models for a specific situation then require extrapolation and specialization to the case at hand. In biology, the search for basic principles and unifying concepts is ongoing, but currently no such fundamentals exist, and their discovery frankly appears somewhat unlikely (in spite of attempts such as the catastrophe-theoretic and field approaches we have considered above) in view of the intrinsic complexity, embodying homeostatic feedback controls and inherent organization, characteristic of biological systems. Of course, the basic physical laws also apply, but constrained by several hierarchical levels of organization that effectively obscure the action of the simple underlying laws in layers of complicated nonlinear interactions.

An immediate consequence of the large numbers of molecules, organelles, cells, tissues and organs present and cooperating in any single biological process is that any attempt at an even moderately realistic or comprehensive model becomes highly complicated and intractable extremely rapidly, while insight into the dominant processes underlying the modelled phenomena tends to be lost in the mass of detail. Any vaguely useful continuous model is thus 'doomed' to be a simplification, and hence a falsification, from the start, thereby losing its philosophical attractiveness as an exact quantitative description of the phenomena in the physical, reductionist sense; so that we need not necessarily go to the effort of developing a 'realistic' model in the first place. Besides, as already clear such detailed modelling attempts are generally premature due to our lack of quantitative knowledge of the underlying biological processes.

Our experience so far of continuous models (consider for example the Oster-Murray models for mesenchymal morphogenesis) has also taught us that biological models, in addition to needing considerable simplification to obtain any useful insights on the effects of different processes and mechanisms on a particular developmental situation, are invariably highly nonlinear and hence analytically intractable. An exception is the understanding we may obtain through dispersion relations and behaviour just beyond bifurcation, and the non-trivial, though admittedly limited, knowledge available for reaction-diffusion systems (see appendix A, and [5, 40, 101, 330] and other works).

With these limited exceptions, however, the study of developmental models inevitably involves numerical discretization of the differential equations on some spatial grid and finite time

differences, and computer solution or simulation (see appendix A.5) which frequently requires lengthy and complex computations, involving vast memories and high-speed computers. The upshot of this is that the analytic advantages of continuity are immediately lost once numerical methods are applied, while the length of the computations furthermore makes it difficult and time-consuming to explore the ranges of behaviour and parametric dependencies of the model. (Admittedly, great benefits have been attained from lengthy computations in physics; but as discussed, the sounder theoretical basis of the models for which complex and expensive computations are carried out renders these *a priori* more worthwhile and justifiable. In biology, model equations are as yet simply too approximate and speculative to justify the effort and expense of extensive calculations.)

We have already had cause to note — see for example section 4.3.5 — that a continuous formulation is not necessarily always the most appropriate in biology anyway. The differential equations are frequently a continuous approximation of a fundamentally discrete process. This is true due to the intrinsic spatial compartmentalization of biological systems, through intracellular or tissue boundaries, but particularly through the discrete nature of cells. Similarly, genes and molecules are countable, discrete entities, and a continuous description depends on an averaging process which may lose some of the details of the behaviour (see [296]).

For the variety of above-mentioned reasons, a discrete dynamical description of development may in many situations be the appropriate one. Such an approach is fairly recent and undeveloped (with the exception of course of discrete numerical simulations of continuous models), so we will only consider it comparatively briefly (relative to our discussion of continuous models), attempting to point out some important features and implications of a discrete approach to modelling.

6.2.1 Cellular Automata

Discrete dynamical systems, or cellular automata, have become increasingly popular in recent years for the study of physical and, more recently, biological systems. Their potential for complex behaviour in spite of very simple fundamental component parts has been recognized, and they have stirred great interest as paradigms for the study of complexity, and as tools for the investigation of the complicated time evolution of physical systems, in particular for simulations in computational fluid dynamics (CFD) — for general introductions, the articles by Wolfram [371, 372, 373] are useful. Applications to biology have come into their own in recent years, as surveyed in a recent review by Ermentrout and Edelstein-Keshet [97].

Introduction Cellular automata, in their simplest form, consist of a discrete number of 'cells' arranged, for example, in a row or two-dimensional grid. The state of a cell is represented by an integer; each cell has some initial state, and its dynamical behaviour is governed by a transition rule, which assigns a new state to each cell based on its current state and the states of its neighbours. Such systems are extremely easy and quick to implement and simulate on a computer, enabling a rapid and flexible study of the behaviour of cellular automata, as typically the rules are much simpler (especially by virtue of being explicit time evolutions) than those found by discretization of a differential equation. Whereas in the numerical solution of a continuous model, great care needs to be taken to overcome spurious effects due to discretization,

in cellular automata the discrete nature of the system is considered a positive attribute, and not disguised, enabling the considerable improvement in speed.

Cellular automata have been around for a few decades, having been introduced by von Neumann in the study of the logical organization of self-replicating structures, with intended overt application to the question of the self-reproduction characterising biological life (see [313] and [106, chapter 21]). They were popularized largely by Gardner in the early 1970s on the basis of John Conway's "Game of Life" (see [106, chapters 20–22]). This simple two-dimensional automaton has only two possible states: alive or dead; and a few simple rules, governing the state of a cell at time $t+1$ in terms of its own state at time t, and those of its eight neighbours. A 'dead' cell will come alive at time $t+1$ if exactly three of its neighbours are alive at time t; whereas a 'live' cell will die in the time step if fewer than two or more than three of its neighbours are alive. These two simple rules suffice for the Life cellular automaton to display an amazing variety of behaviour, depending on the initial configuration, that includes the generation of very complex structures and universal computation. But it is only in the last decade or so, since high-speed computers have become freely available, that the study of cellular automata has flourished and come into its own.

Basic Characteristics of Cellular Automata

The fundamental defining characteristics of the traditional, basic types of cellular automata have been classified by Wolfram [373, p.vii] as follows:

- They consist of a discrete lattice of sites;
- They evolve in discrete time steps;
- Each site takes on a finite set of possible values;
- The value of each site evolves according to the same deterministic rules;
- The rules for the evolution of a site depend only on a local neighbourhood of sites around it.

Some of these general requirements may be weakened or modified, as we shall see later; in general a cellular automaton consists of *a simulation which is discrete in time, space and state* [97]. These simple defining properties permit the great variety of behaviours of cellular automata, both in terms of discrete idealizations of partial differential equations, and in their own right; for, as discussed above, cellular automata do not necessarily merely provide an approximation of partial differential equations such as reaction-diffusion equations, but may form an independent 'caricature' of the 'true' physics [202]. There is also a deep connection to computation, which we shall touch on later.

As already indicated, cellular automata may be considered as discrete dynamical systems, which in general display irreversible dynamical evolution. Thus trajectories on the configuration space for cellular automata eventually tend towards *attractors*, as for continuous systems. Such evolution towards complex and structured attractors from arbitrary initial states may be considered as 'self-organizing' behaviour, the formation of structure from structureless initial states.

This provides the motivation for the study of automata in the generation of developmental complexity.

Attractors of Cellular Automaton Evolution Extensive, mainly empirical studies have been performed of the possible solution behaviours of cellular automata; these have shown that the attractor topologies and patterns take on four qualitative forms, corresponding to four distinct universality classes, the first three of which correspond roughly to attractors of continuous dynamical systems [371, 374]:

Class 1 *Spatially homogeneous state* (corresponding to a limit point) — here the final configuration is *totally predictable*, and independent of the initial state;

Class 2 Sequence of simple *stable* or *periodic structures* (analogous to a limit cycle or multiply periodic solution) — the local behaviour of the final state is predictable from a *local initial state*;

Class 3 *Chaotic aperiodic behaviour*, with perturbations growing indefinitely at a fixed speed (corresponding to strange (chaotic) attractors of continuous systems) — the configuration depends on an *ever-increasing initial region*;

Class 4 *Complicated localized structures*, some of which are propagating — the behaviour is *essentially unpredictable*.

The behaviour of this fourth class appears to have no counterpart in continuous dynamical systems, and represents a 'higher level of complexity'. Thus on this basis cellular automata would appear to be more suited than continuous systems for the study of complexity and self-organization. Indeed, there has been a surge of interest in this field, which is far too vast for more than a mention, and at least one journal, *Complex Systems*, is primarily devoted to cellular automata research. The popularity may also be ascribed to the fact, already alluded to, that although the behaviour is complicated, it may be very easily generated by computer simulation, and attractive and striking space-time patterns may be produced, given for example appropriate choices of colours for the different states.

The above studies of the qualitative behaviour represent possibly a first step towards formulating general theories of cellular automata, and a wide variety of analytical methods has been applied to their study [371]. These include the statistical and thermodynamical properties of the configurations — essential in the face of the otherwise impenetrable complexity — including entropies and dimensions; consideration of information content and propagation, including the rate at which disturbances are transmitted through the medium; and the use of computation and formal language theory to give a more complete characterization of the self-organization of cellular automata. One result is that some cellular automata, including the "Game of Life", have been shown to be capable of *universal computation*; that is, they can implement any finite algorithm, and thereby emulate the behaviour of any possible computer and hence (since any physical process can be represented as a computational process) of any physical system as well. General class 4 cellular automata have been conjectured to be capable of universal computation [374], rendering them capable of arbitrarily complicated behaviour and giving them a complexity beyond that of any dynamical system (see also section 3.1.5).

Motivation for the Study of Cellular Automata in Development

Computational Irreducibility and Parallel Processing A consequence of the computational universality is that such cellular automata are computationally irreducible. That is, there is no algorithm or more efficient procedure for determining the outcome of the cellular automaton evolution than the explicit simulation of each step, so that the attractors are *logically deep* objects [28]. In simple cases (for example in dynamical systems converging to a fixed point or limit cycle attractor) a mathematical formula for the overall long-time behaviour of the system is available; such computationally reducible phenomena are the ones traditionally studied in the physical sciences. However, for cellular automata of classes 3 and 4, with chaotic or irregular attractors, explicit simulation in a computer experiment may be the only method of investigation. This may well correspond to many real systems, such as fluid turbulence, which are probably computationally irreducible; and a conjecture is that the form of a biological organism can also essentially be determined from its genetic code only by following each step in its development [372]. That is, organismal structure displays computational irreducibility, and can not be predicted uniquely from the genetic and zygotic information, only traced through development — development is fundamentally a *process*, which must ultimately be followed through in order to discover the final structure.

Such considerations by no means invalidate all our attempts at finding underlying mechanisms and obtaining knowledge about the forms that may be produced, through the establishment of models and the solution of dynamical equations, however. After all, the development of different individuals of the same species is, on the whole, quite similar and highly reproducible. The possible forms available to developmental mechanisms are *constrained* by the available genetic information and epigenetic mechanisms utilized by the developmental process. But there is always the stochastic element, the dependence on detailed initial conditions, the interplay between chance and determinism in self-organization, which dictates the detailed outcome of any developmental process, and this can never be predicted, only observed at the end.

We thus have a fundamental philosophical motivation for the use of cellular automata for the study of biological complexity, and in particular in development. Their attractiveness is increased when the parallel-processing nature of cellular automata is considered: computers process information serially, whereas in a physical or biological system, there are no lengthy mathematical calculations, just the continuous response of the system at each point in space and instant in time, constrained by physical laws and the various forces and other influences acting on it. Such behaviour is emulated by the cellular automaton used to model a physical system; the values of all the cells are updated together at each time step (of course, with current computer architecture a serial algorithm is still needed to simulate this fundamentally parallel process [372]). Thus, potentially far more than a differential equation formulation, where under suitable conditions properties of the solutions may be deduced from the form of the equations, a cellular automata formulation may well constitute the appropriate language for development, capturing its essential nature as a fundamentally *parallel process.*

Cautionary Comments On the other hand, the complexity of biological structures results at least in part from intricate hierarchical and feedback interactions among the multitude of structures and species at different levels of organization, and it is not clear how complicated structures may be captured in a description that gains its strength precisely from the application

of simple rules and the use of a small number of states and discrete cellular entities. Once more complex rules are created, the cellular automata advantages of speed and conceptual simplicity are in danger of being lost; thus one should be careful not to push this paradigm beyond its inherent strengths [97]. The challenge is to take full advantage of the opportunities afforded by cellular automata, as exemplified by the appropriateness of such a parallel-processing, computationally irreducible description as discussed above, in the study of biological complexity and self-organization, while still somehow retaining the simplicity of the description. It is not clear to what extent this is possible; the applications to biology in general, and developmental biology in particular, are in any case still in their infancy (for a survey see [97]). Ultimately, cellular automata complement other descriptions; understanding is best served when one has available a hierarchy of models, each providing insights at its own level of detail [202].

More General Cellular Automata

One objection that might be raised concerns the deterministic nature of cellular automata as defined above; can this be reconciled with the stochastic perturbations constantly buffeting the embryo? In fact, the deterministic automata, as studied by Wolfram and exemplified by the "Game of Life", form only one class of cellular automata, a class in which the spatial domain of the model is divided into a fixed lattice and each lattice point has an associated state, determined solely from the earlier states of the cell and its neighbours. Such automata are closest to forming a discrete approximation to differential and integral equations, as discussed above. An immediate extension to such deterministic automata is then that random noise may be introduced directly into the cellular automaton rules, simulating environmental perturbations [373]. Such probabilistic cellular automata are found to exhibit features analogous to phase transitions, as a function of noise level.

Ermentrout and Edelstein-Keshet, in their recent survey [97], have classified cellular automata into three broad classes. Their first class, the *deterministic* or '*Eulerian*' automata, corresponds to those we have already considered above. To extend these, not just the requirement for determinism, but also that for fixed spatially-distributed cells may be relaxed. Thus we obtain the second class, denoted *lattice gas* models, or *particle systems*; such automata consist of a discrete spatial grid on which particles move about, usually driven largely by random events, and interact in some prescribed fashion. Hence the same initial conditions will not yield identical final states except in some average sense, providing a plausible paradigm for biology. The final class consist of so-called *solidification* models, which are much like lattice gas models except that once a particle is in a 'bound' state, it is fixed and can never move or disappear again; this is used to describe various growth and aggregation processes. With these extensions, cellular automata provide a broad palette of possibilities for the simulation of biological organization, and we shall proceed to explore below some of the applications that have been proposed.

6.2.2 Cellular Automata in Development

Cellular automata potentially provide a new paradigm for the study of development, as we have seen. In spite of this, there does not appear to have been much application of these overtly promising concepts to the study of developmental pattern formation, especially from a self-organization perspective, and we are limited to discussing the rather few discrete models that

have been proposed. The prolific and productive use of cellular automata in biology is hampered by the considerable difficulty of choosing appropriate rules and state spaces for the simulations; for ultimately the search is for *understanding* of self-organizing processes, not for the generation of suggestive pictures.

Two general approaches may be used to derive cellular automata to apply to development (or any other field) [202]: the simplest is to generate heuristic automata, 'working backwards' and abstracting the observed phenomena into a few simple rules that reproduce the observed structures and processes, without attempting any explicit analogy to a mathematical model based on some underlying mechanism. Such a trial-and-error approach has drawn criticism for having 'arbitrary' rules, from which insight into mechanisms may not easily be deduced, and hence being unrelated to biological systems. Frequently the structures that are produced in computer simulations are already latent or explicitly incorporated into the formulation of the computer model, not novel or self-organized. On the other hand, one might assume that when a simulation matching observed patterns is found, a logical structure in the underlying mechanisms has been unearthed, so that one may proceed to seek or speculate on the basic mechanochemical mechanisms corresponding to the 'successful' rules or simulations [384].

The alternative approach is to derive an automaton directly through the discretization of a realistic mathematical model grounded in the consideration of biological mechanisms; this can clearly correspond to the numerical solution of differential equations, in which case the space and time discretizations are essentially obvious, but the finite state space of automata is often difficult to assign. Rather than going via a continuous formulation, one can also construct an automaton from first principles, and attempt to endow it with features that correspond to the physical interactions being simulated. As before, as an introduction to the problem of biological self-organization, the first successful application of such an approach we present is to the paradigmatic chemical example of self-organization, which we have already considered in depth.

The Belousov-Zhabotinskii Reaction

Excitable media (those in which small perturbations decay to the unique stable rest state, but perturbations above some *threshold* undergo a large transient excitation before entering the recovery phase and relaxing to the rest state, such as the BZ reagent) frequently have a description in terms of partial differential equations, as seen above (see appendix B), but extensive numerical simulations of such systems are difficult and costly, and singular perturbation analyses have limited validity (see appendix A.3.3). Thus there has long been an interest in discrete simulations for exploring the temporal evolution of excitable media [109]. In such a cellular automaton approach, each element can exist in one of three states, resting ('quiescent'), excited or refractory ('tired'); and these states may change in discrete time steps according to the states of the neighbours. Such models have been popular for their intuitive simplicity and appeal, and for their ease and speed of computational implementation. Thus there have been several applications of cellular automata to the study of the BZ reaction and other excitable media, such axonal excitability and cardiac fibres; for an introduction to typical rules used in models of excitable media, see [97].

We have noted in some depth the peculiar pattern-forming characteristics of the Belousov-

Zhabotinskii (BZ) chemical reaction, which displays striking spatio-temporal structures such as concentric and spiral waves and three-dimensional patterns (see appendix B for a detailed introduction). A reaction-diffusion model, the 'Oregonator' [100], isolating the significant features of the kinetics, and displaying solution behaviour that captured the major observed features, was also presented, illustrating the power of the kinetic, dynamical systems approach to the study of self-organization in such a real experimental system. More recently, however, interesting features of the autocatalytic chemical reaction systems have also been simulated by discrete cellular automaton models, implying some form of contact-mediated interactions between cellular entities in the system rather than long-range diffusion as the trigger for the spatial symmetry-breaking and patterning. (See for example the popular article by Dewdney [79], which describes the 'hodgepodge machine', a cellular automaton capable of simulating a variety of patterns observed in the BZ reaction.)

Simulations of the BZ Reaction Early simulations were produced by Madore and Freedman [213], who introduced an algorithm operating on a hexagonal grid, with simple rules and two parameters, one corresponding to the productivity of the reaction on a local scale length, or probability of propagation, and the other the delay or quiescent time after the localized reaction. Cells could either be 'activated' ('excited'), or 'quiescent' ('receptive'). The time evolution of the algorithm produced basic morphological features remarkably similar to those observed in the BZ reaction, in particular self-organized wave structures such as single- and multi-armed spirals. This work demonstrated that globally coherent structures could appear spontaneously without the need to invoke either diffusion or putative 'local oscillators'. Here, however, the matching between the rules and the BZ patterns was heuristic — the algorithm used was originally studied in the context of self-propagating star formation. A similar model of an excitable medium has been studied by Winfree and coworkers, utilizing a cubic three-dimensional grid of cells to obtain three-dimensional waves such as a scroll ring and a linked pair of twisted scrolls [370].

Increasingly complex rules have been used to obtain quantitative agreement between the simulations and experimental or more realistic numerical results (the latter obtained from calculations on the Oregonator). Recent work by Gerhardt *et al.* [109], for example, is able to include two critical effects of wave propagation in excitable media, namely curvature and dispersion (see also [79]). By introducing two state variables into each cell, corresponding to an 'activator' which can take two values, and an 'inhibitor' which is permitted a range of values, and by allowing spatial averaging over several cell distances, convincingly realistic pictures and quantitatively correct dispersion relations and spiral curvatures are obtained. Markus and Hess [221] obtain similarly curved spirals; rather than introducing complex rules, their approach is to allow long distance interactions on a random spatial grid, as a means of generating isotropic cellular automata (as in the usual square or hexagonal automata, the grid anisotropies tend to be propagated into the patterns produced). The derivation of such cellular automata may also proceed through the discretization of a reaction-diffusion system, with finite discrete ranges permitted for the state variables. The reactions used to model excitable kinetics are typically linear caricatures of sigmoidal interactions with decay and autocatalytic self-excitation — this technique corresponds effectively to the approach of Gerhardt *et al.* [109].

Thus our paradigmatic self-organizing system, the BZ reaction, may be presented as an excellent example of the utility of both the continuous dynamical systems modelling approach, as exemplified by reaction-diffusion equations (see appendix B) and the discrete, cellular automata

technique. In both cases, the self-organization potentialities of the dynamics are clearly brought to the fore. The successful quantitative modelling of the BZ reaction — and by extension, of most self-organizing systems — will in all likelihood rely on a combination of the two major paradigms, the continuous and discrete approaches. We continue with applications of cellular automata to pattern formation in developmental biology, and again refer to a system that we have much studied using various continuous dynamical models — integumental patterns.

Discrete Models of Integumental Patterning

The most obvious and striking patterns in biology are surely those that adorn a multitude of animals, with particularly familiar but nevertheless remarkable features being the often colourful stripes and spots on animal coats, bird and butterfly wings, and shells. The variety of models we have studied for such integumental patterns encompasses almost the entire range of mechanisms that have been considered in this thesis. Thus it comes as no surprise to find that here, in the concluding set of models, applications to integumental patterns again feature strongly. The results of a number of cellular automaton simulations have borne a striking resemblance to the intricate patterns of growth and pigmentation observed in biology.

Shell Patterns An early simulation by Waddington and Cowe [359] attempted to reproduce the formal characteristics of molluscan shell patterns by computer simulation. The rules in this case were purely heuristic and formal, and chosen to produce an acceptable simulation, although an attempt at a physiological justification in terms of deposition, self-reinforcement and destruction of a pigment precursor was made. A fair simulation of the lines of pigment on the shell of a mollusc of the genus *Conus* was obtained, but otherwise the rules were of dubious validity and the range of possible patterns limited; thus the model should be seen merely as an introduction to the *potential* of discrete simulations, when endowed with appropriate rules.

We have noted that in the ensuing years, two major approaches to shell patterns, linked conceptually through their implementation of local activation and lateral inhibition, have been introduced: the neural model of Ermentrout *et al.* [96] (see section 5.1.4) and the reaction-diffusion model of Meinhardt and Klingler [236] (see section 4.3.2). Discretization of either of these models will clearly lead to a cellular automaton, which will display similar pattern-forming properties to those demonstrated (through discrete numerical simulation!) by their continuous analogues. The neural model, incorporating an integral equation formulation and long-range interactions, of Ermentrout *et al.* (discussed also in [251]) is particularly amenable to a discrete formulation, as in its original formulation it already contains a discrete time variable. Thus the patterns on molluscs form an appropriate arena for the demonstration of the potentialities of cellular automata [97].

Animal Coat Patterns: a Local Activator-Inhibitor Model As we might expect, animal coat patterns are similarly suited for such an application. As an alternative to the Turing-type continuous reaction-diffusion models proposed by Murray [248] and Bard [14] (see section 4.3.2), Young proposed a simple spatially discrete model to generate vertebrate skin patterns [383] (for a brief discussion, see [87]). He assumed a distribution of pigment cells of two types, differentiated and undifferentiated, and in the manner of cellular automata simulations let the differentiation

state of each pigment cell depend on the sum of influences around it. These influences were assumed to be due to a field of activation surrounding the cell at radius R_1 and inhibition at larger radius R_2; the net effect on a cell was found by spatial averaging with a threshold weighting function, and if this net effect was positive, the cell would differentiate into a pigmented cell. The initial conditions of the calculation were random distributions of differentiated cells on a rectangular grid; the morphogenetic fields were then summed and the differentiation states changed according to the rules until the resulting pattern stabilized. Generally, very few (about five) iterations sufficed for convergence (in considerable contrast to the situation for numerical solutions of partial differential equations!).

The general form of the final pattern was found not to be sensitive to the initial distribution of differentiated cells, but it depended on the intensity of inhibition and activation, and the sizes of the respective neighbourhoods. With decreased inhibition, a spotted pattern connected up into a pattern of stripes, indicating a close relationship between spotted and striped patterns, so that fundamentally the same mechanism could produce both patterns in vertebrates. The ability of such a simple simulation to produce patterns similar to those observed, and those produced by reaction-diffusion models, again suggests some congruence between the models and the mechanism utilized in nature. Note especially that the cellular automaton model explicitly embodies the features of short-range activation and long-range inhibition constituting the lateral inhibition basis which we have found to be fundamental to a diversity of self-organizing mechanisms [287]; thus the similarity between these patterns and those produced for instance by a reaction-diffusion mechanism is due to the underlying logical structure, which is made quite explicit here. Possible mechanisms for the activation and inhibition are suggested to be the short-range diffusion of morphogen molecules, but this is not crucial to the model, and direct cell contacts or local elastic strains could just as well form the processes at work; of importance is the *conceptual* basis of the model, which provides a good example of how a cellular automaton simulation can illuminate the *minimal requirements* for a particular type of pattern formation.

Local Cell-Cell Interactions A somewhat different study of discrete aspects of pattern formation has been made by Cocho *et al.* [59, 60, 61]. Local cell-cell interactions, through attachment of cells to each other by cell adhesion molecules, were taken as a basis from which 'physical-like' scenarios about the 'freezing' of cells into a fixed configuration in the presence of conflicting cell-cell and cell-background interactions, were derived. Two possibilities were considered: the first [59] assumed the time evolution to be slow enough that cells could accommodate a global minimum energy configuration consistent with the conflicting constraints. For this case, a phenomenological 'energy' function was defined and minimized on a lattice, and some simple patterns were obtained that were compared to those found on some snakes, mammals and lizards.

The second model [60] assumed that equilibrium was not attained, as pattern formation was too fast to achieve a global minimum energy; in this case, a clonal cellular automaton model was proposed, in which each row or sheet of cells, already 'frozen' into its pattern, acts as the initial condition for a cellular automaton type of growth in which the pattern of successive rows depends on interactions with those already established, by analogy with unidirectional solidification of metals. Simple square or triangular lattices with two states were used to simulate a range of patterns, again related to those on snakes, felines and some fish. Minimal energy accommodation of nuclear-nuclear interactions was also cited as a possible mechanism for the formation of

Drosophila bands [61]. The rules in these simulations, although purportedly related to aspects of homotypic and heterotypic binding through cell adhesion molecules [81], were largely heuristic — the effects of different rules were explored and compared to observed patterns. Of interest in this work, however, is how it combines kinetic, dynamical properties such as through the cellular automata, with equilibrium considerations of minimum energy configurations of adhesive interactions [150].

Other Applications of Cellular Automata to Development

We may continue to consider the two other classes of automata in the classification of Ermentrout and Edelstein-Keshet [97], as some benefit for developmental questions may also be gained from the use of biological 'lattice gas', and growth or 'solidification' models. We have already seen an application of the first of these (see section 5.1.3), to the question of the orientation of fibroblast cells into parallel arrays through contact-mediated interactions and realignment in fibroblast aggregation [89, 90]. Here a continuous, integro-differential equation model was established to account for the reorientation, and analytical studies of the continuum model, but ignoring spatial distributions, showed that a symmetry-breaking instability (in angular directionality) could occur, so that cells could spontaneously align. However, due to the considerable analytical complexity that would have arisen from an attempt to include the full spatial behaviour and motion into the continuum model, the spatial alignment of cells into parallel arrays was rather demonstrated with the aid of cellular automata simulation (see [89, 97]). The simulation demonstrates the result predicted from the local continuous analysis, namely that for cell density below a certain critical value, the distribution of orientations remains isotropic and transient alignments die out, while above a critical density, parallel arrays quickly form. This thus provides an example of how an analytically intractable continuous model may frequently succumb readily to discrete, cellular automaton simulation.

The final class, of 'solidification' automata, that may be viewed as models for growth in a medium, will be considered in more depth later, as there are numerous implications for the geometry of the structures that may be formed, as well as for the iteration of rules and algorithms. A limited range of other applications of cellular automata to developmental biology is available. We have already encountered various discrete simulations, for example for cell sorting [338] (see section 5.3.1); such models are based on the differential adhesion hypothesis and again on the minimization of energy functions. An extensive discussion of such work is given in the book by Goel and Thompson [118], who note that many phenomena involving cellular rearrangements may be simulated, especially when flexibility in cell shape and motion is incorporated [338]. Further simulations have been presented by other authors (for references, see [97, 118]), but in general rules suited for the production of the final patterns are *chosen*, and the simulations bear little relevance to issues of self-organization.

6.2.3 Iterative Rules in Development

Before we proceed to investigate some iterative, rule-based discrete automaton systems, it is relevant to consider briefly the possible nature of the rules that we are considering; for as we have seen, the complete specification of a cellular automaton requires the introduction of a set of rules according to which the new state of a cell may be obtained from its previous state and that

of its neighbours. The units of cellular automata thus essentially behave like miniature robots, executing iterative subroutines; to what extent do embryonic cells satisfy this description?

A Developmental 'Program' The concept of a developmental 'program' is popular and controversial [280, 364]. The elucidation of the genetic code and the molecular biology of transcription and translation, together with the ample evidence for heredity and the developmental damage caused by mutations, has bolstered the view that the genome contains a 'program' which must merely be 'run' in order to obtain an organism (in this cybernetic metaphor, "genetic engineering is analogous to computer hacking"! [63, p.14]). The computer metaphor in developmental biology has produced some valuable insights, for instance enabling useful interpretations of the status of some genes, such as those containing a homeobox or zinc finger motif in their coding sequence, which regulate the transcription of other genes and thus play a primary regulatory and directive role in some developmental systems (see also sections 2.1.1 and 2.2.3). Indeed, in systems such as the early development of *Drosophila*, a fixed sequence of gene expression patterns and feedback-controlled regulatory interactions appears to play a primary controlling role.

On the other hand, as we have seen throughout this thesis, there are many systems where the relationship between genes and developmental processes is not *causal*; where morphogenetic processes are the consequence of the coordinated interactions of cells utilizing molecular and cellular properties at a hierarchical level of organization well above the genetic level. By coding for proteins such as cell adhesion molecules and the structural proteins of the cytoskeleton, genes are *necessary* (but not sufficient) for successful development, and may be said to *permit* morphogenesis by creating an appropriate milieu; but ultimately development is the outcome of a combination of genetic and epigenetic ('generic' [268]) factors, involving chemical interactions and physical processes, whose interaction is frequently *self-organizing* — the proper recognition of this is the major concern of this thesis. In the overall scheme genes tend to play a permissive, rather than a prescriptive, directive role; and the genetic underpinnings of some given morphogenetic movement or structure formation will probably more often than not be found to be diffused over the entire genome. Thus the statements 'genes control development' or 'the genome contains a program for development' are, in general, not very helpful. For a fuller discussion of the limitations of the concept of a developmental program, see [125, 280, 364]. (Held takes a generally opposite position, presenting a comprehensive discussion of the utility of the computer metaphor in developmental biology; he considers aspects such as combinatorial coding in hierarchical specification of states, iteration and halt conditions and modular organization in showing how the seemingly automatic nature of development may be likened to computers [159].)

'Rules' in Development Unless they are abstracted from some specific physical or chemical interaction (as in recent cellular automaton studies of the BZ reaction), the nature of the rules employed in discrete simulations is generally left unspecified. As we have noted above, both genetic and physicochemical processes suffice to have the marked effects reproduced in the simulations. Thus, for instance, the rules could correspond to individual genetic instructions, or more likely to *subroutines* — in computer terms, sequences of algorithms or instructions that may be accessed by a single command. Such subroutines may be composed of groups of genes, which are functionally if not spatially coupled (although the colinearity of some insect

and vertebrate homeobox-containing genes, positioned on the chromosome in the same order in which they are expressed, provides evidence even for spatial organization of the genome); these genes may be coordinated through a common promoter region (which could be regulated by the gene binding domain encoded in the homeobox), or be coupled as a cascade expressed in sequence. As the same set of instructions may be used repeatedly in the development of different parts of an organism, the use of such DNA subroutines affords economy of genetic specification and efficiency.

Alternatively, our experience in the study of mechanisms and particularly self-organization has shown us that a single 'instruction' can be sufficient to trigger off the complex coordinated activity that constitutes a certain physical or chemical morphogenetic mechanism; for instance, an increase in traction may lead to condensation in mesenchymal morphogenesis. Then the automaton rule is just a discrete expression of some physical or chemical interaction, corresponding possibly to mechanical forces or energy-minimizing constraints. In either case, genetic or epigenetic, complex coordinated activity may result from the carrying out of a 'rule' such as that postulated in cellular automata. Of course, the effect of calling a subroutine, or carrying out a rule, depends on the *context* within it occurs; for instance, chondrogenesis may be assumed to utilize generally invariant rules and mechanisms, and it is the different environments and constraints, with varying initial and boundary conditions, that are responsible for the differences between, say, an arm and a leg (see especially also section 2.3.3). We may proceed to consider some automaton simulations fundamentally based on iteration, in the light of these considerations of the possible meaning to be ascribed to iterative 'rules'.

L-Systems

A class of mathematical models that purported to explain major aspects of development and generated much interest, especially in the early 1970s, was that of L-systems, invented by Lindenmayer. This approach involved the construction of formal mathematical systems in which multicellular organisms were construed as arrays of symbols, or finite automata, each standing for a cell. Their development was modelled by *algorithmic rules* which provided for the substitution of new arrays for each symbol in the previous array, these replacements, the *tasks* of the cell, corresponding to cell division or death, or changes of cellular state.

These rules could be quite complex, and depended in general on the previous state of the cell, so that this mechanism depends on lineage [159]; a cell was born with a *task selection rule* [224], used both to determine its task assignment (using information depending in general on positional signals; the orientation, presence or absence, and task status of neighbours; lineage and task of parent; position in developmental tree and similar factors) and the task selection rules of its progeny. The general scheme provided both for internally-derived and externally-specified information; in addition, growth was specifically incorporated. Thus the models and transition rules combined a stem-cell type of growth and internally-motivated state changes.

On the basis of such assumptions on the types of permitted arrays and algorithms, an entire class of mathematical models within the framework of formal language theory was developed and studied, particularly by Lindenmayer. The specific motivation was to cellular behaviour in development, and this was studied both with [206, 207] and without [208] cellular interactions; but the resultant body of results was rather abstract, concerned more with the formal properties,

the languages and grammars arising out of such models, than with many practical biological applications. A particular restriction was the usually one-dimensional nature of the simulations. The applications have tended to be restricted to plant, fungal and other protistal, and bacterial systems, and have focussed on aspects such as the shaping of leaf margins, the development of sexual reproductive structures in fungi, and heterocyst distributions in cyanobacteria (blue-green algae); there is little relevance to animal development.

Useful surveys of L-systems and similar automaton-theoretic models of growth and development are given in [209, 224], and in the collection of articles edited by Lindenmayer and Rozenberg, [210]. The interest in such string rewriting systems for development appears to have waned in the last decade; while rules that account for certain one-dimensional and branching patterns may be obtained, such an abstract approach (of interest, of course, for its formal structure and grammars) has clearly not provided much insight into developmental mechanisms. Recent interest in such theoretical representations of branching patterns is exemplified by work published in 1991 on speciation in red algae [242].

Other Growth Automata

Young and Corey [384] have investigated applications of simple iterative rules for growth of cells on a two-dimensional lattice, and have shown that these can simulate some aspects of the growth of fern gametophytes, branching fungi and leaves realistically. No attempt was made at deriving a realistic model, using for instance continuum-mechanics techniques; rather, a more or less *ad hoc* scheme was used. Trial-and-error simulations were used on random cellular automata, and when the resultant shape was found to mimic a biologically observed pattern, it was assumed that the growth rules were endowed with a logical structure corresponding to that in the actual biological process, although this structure might be well hidden in the biochemical details. Isotropic rules produced uniform growth, as expected, while suitably anisotropic growth rules were able to simulate nonuniform growth in different directions. Changes in the growth rules were also able to produce allometric distortions and variations among simulations, comparable to the coordinate transformations that could map the geometry of one species onto another, as pointed out by D'Arcy Thompson [348]. Other rules were applied to simulate branching and leaf growth.

The intention of this work, and others like it, was to generate the simplest model that could capture the essential features of an observed phenomenon, and hence the logic underlying it; in the recognition that the immense complexity of biophysical phenomena makes attempts at exact simulation and mathematical analysis unrewarding and, at present, quite unfeasible. The hope was that the accurate modelling of growth and the stating of explicit rules could lead to some understanding, as the appreciation of the underlying logical structure may indicate what to look for in the search for mechanisms.

Studies of fungal branching and growth of bacteria constitute the biological applications of 'solidification' automata noted by Ermentrout and Edelstein-Keshet [97]. Thus, for example, a continuum model for the two-dimensional branching and cross-linking involved in the formation of networks such as fungi [88] has been simulated by a simple discrete automaton with simple growth rules [97]. A difficulty all with these applications of growth automata, such as iterative rules and L-systems, for our present purposes is that they are largely concerned with growth

and branching, and are thus more suited to studies of plant and fungal development, as we have noted; whereas our overall focus has been towards the study of mechanisms of animal development (see section 2.3.1).

6.2.4 Fractal Structures and Chaos

The models of branching and lattice growth automata models discussed above have little direct application to vertebrate patterning, and also ostensibly tell little about self-organization, although they do provide an important example of the third kind of biologically-motivated cellular automata [97]. But they lead us on to the discussion of an important final point, that of the status of *chaos and fractals* in developmental biology. These topics, which conclude this thesis, are included here as they are somehow intermediate between discrete and continuous dynamical system formulations: they have deep connections both to the repeated application of rules, and to continuous differential equation formulations of dynamical systems.

Fractals

The repeated iteration of the same transition rules or subroutines, possibly on different scales, can lead to recursive cycles in the formation of branched networks. Consequently, structures on different scales may be similar, that is, the overall system displays *self-similarity*. This is the essence of **fractal** structures, which are composed of similar subunits at ever finer levels of detail.

There has been considerable theoretical and public interest in fractals, as evidenced by the popularity of the Mandelbrot set, possibly one of the most attractive and best-known mathematical objects (see the colourful book by Peitgen and Richter [304]). We shall not here dwell on the mathematics of fractals, including important questions of how to calculate their dimension (the traditional concept of dimension is meaningless for fractals, as they have structure on all scales, and must be replaced by more generalized definitions such as the Hausdorff dimension — which is fractional for fractals, hence their name), referring to any of the texts or papers referenced in this section for an introduction (see especially [21]).

Self-Similarity and Fractals in Development The concept of the recursive application of a developmental subroutine or subunit of the DNA to produce biological structure has become fairly widespread, by the repeated application of rules of relatively few elements. This suggests a possible explanation for efficient storage of information to create complex spatiotemporal organization of cells during development — this indeed is possible for all physical mechanisms which are driven by epigenetic factors, but here the structure might even be completely genetically specified, and still require relatively little coding.

Computer-generated pictures, based on fractals, which remarkably resemble biological objects such as ferns and trees have caught the popular imagination. For striking and colourful simulations, together with a general course in deterministic fractal geometry and an introduction on the use of fractals to model objects in the physical world, including biological systems — by the search for a specific fractal to fit a chosen natural object, rather than from first principles — see the book by Barnsley, whose title, *Fractals Everywhere*, echoes this recent trend [21]. (A

further class of nonequilibrium growth models, with simulations generating fractal-like structures, is presented by Meakin [228]). It should be noted firstly that the self-similar structure apparent in biology penetrates down only a few levels, whereas a theoretical fractal has infinite detail of structure down to infinitesimal scales; hence it is largely the *concept* or paradigm of fractals, rather than their strict mathematical definition, that is of interest in biology and indeed, in other 'real world' systems such as mountains, coastlines, clouds and the host of other systems that have been claimed to be fractal. Furthermore, with a few exceptions in animals discussed below, this type of structure occurs only in plants and fungi, which are subject to continuous development and growth throughout their life spans; their morphogenetic pathways, quite different from those of animals, do not overly concern us here.

Fractal Structures in Vertebrates There have however been observations of fractal geometries in some animal organs and structures, which are of more interest to us. Goldberger and West, in particular, have used fractals to analyze and model complex physiological structures. Their studies, and those of others, have indicated that the fractal criteria of *multiple scales* and *self-similarity* appear to be met by a number of structures in the human and animal anatomy, in networks of blood vessels, nerves and ducts [120, 363] (see [119] for a popular introduction). An important example is the tracheobronchial tree of the lung, which shows detailed self-similar branching for 23 levels of structure. Similarly, the vascular system is a branching network of tubes with many apparent scales. Multiple levels of organization, self-similar branching or folding may also be observed in neural networks, the cardiac surface of the heart, the urinary collecting tubes in the kidney, the multiply enfolded mammalian brain, and several other physiological systems [120]. Measurements on patches observed in mosaic liver, for example, have yielded evidence for fractal dimensions, implying an iterating, self-similar process involved in organ development [173].

Such self-similarity in structure must be the remnant of a fundamentally self-similar morphogenetic *process*, which implies as we have seen already a principle of a potentially 'simple' code iterated on progressively smaller scales, underlying the construction of many highly complex, irregular structures. Of course, for each iteration other factors might be brought into play to cause local modifications and fine-tuning at each level, but the crude outlines of the structure being formed require only the repeated application of the same rules or subroutines.

Having recognized that seemingly fractal physiological structures are produced by recursive morphogenetic processes, by mechanisms that are applied at successively smaller scales, we can place bounds on the possible mechanisms that may be at work. For instance, we can eliminate the *need* for individual genomic specification of every detail in favour of repeated usage of a single mechanism; although of course the actual mechanisms are the, frequently 'untidy', products of natural selection, and will thus not necessarily conform to the tempting expectations of simplicity and lack of redundancy. This recognition however tells us no more about the actual *nature* of the mechanism that is repeatedly applied at different levels, and any of the mechanisms and models that we have considered throughout this work, or even totally different ones, are potential candidates.

There could be physical processes — this is likely in the tube formation involved in the creation of the vascular system and bronchial tree — or chemical signalling, genetic or epigenetic mechanisms, or most likely a combination of all of the above, as is prevalent throughout develop-

ment. Fractal structures exemplify complex organization as the outcome of simpler mechanisms, but their creation is essentially peripheral to the issue of self-organization; there may or may not be self-organizing processes at work. The fractal algorithm essentially takes whatever routines are available, with whatever physicochemical and conceptual basis, and utilizes them for its purpose of creating self-similar structure. The search for physiological fractals has provided a unifying theme to diverse complex structures, but the complexity manifested here is of a different nature to that motivating our study of self-organization, being generated not spontaneously but by a recursive rule-based process.

Chaos

A corollary of the fractal structures inherent in physiology is that their processes seemingly embody *chaotic dynamics*, which may be recognized through temporal behaviour with fluctuations over multiple time scales. The most studied system is the heartbeat, where the Fourier spectrum of the heart rate time-series is a broad band for the normal heart, while frequency analyses of arrhythmias such as ventricular fibrillation reveal a narrow-band spectrum, indicating very regular behaviour [119, 120]. Thus the phase space representation for the normal heart beat appears as a strange attractor, indicating chaotic dynamics, and regular behaviour is an indication of disease. Chaos also appears to be a normal feature of other components of the nervous system, as the analysis of electroencephalograms (EEGs) of healthy individuals has indicated. Such chaotic dynamics are surely somehow connected with the fractal physiology created by recursive morphogenetic processes.

Thus we have arrived eventually at the question of **chaos**, the generation of highly complex behaviour in a potentially simple system with few degrees of freedom, and thus the converse of that other counterintuitive feature of nonlinearity, namely self-organization, the creation of structure and order in a complicated system with many components and degrees of freedom (see section 3.1.1). We have seen extensively that self-organization — symmetry-breaking, the spontaneous generation of inhomogeneity and structure — is a highly fruitful and useful concept in developmental biology; what about chaos?

The scientific and public imagination has been captured in recent years by the concept of chaos, which is characterized by unpredictability in completely deterministic systems, by sensitive dependence on initial conditions, by the exponential amplification of arbitrarily small effects, by persistent instability. We have no space here to pursue this fascinating and hotly studied field any further; for popular introductions, see for example Gleick's book [117], and the *New Scientist Guide to Chaos* [144], which contains reprints of a range of introductory articles, while the book by Schuster [318] is one of many more detailed introductions.

There are few explicit attempts to incorporate chaos into a theory of development. One such attempt is made by O'Shea [285], who wishes to use Hamiltonian dynamics and KAM (Kolmogorov–Arnol'd–Moser) theory to account for periodicities such as the creation of the early segmentation pattern of the *Drosophila* embryo. Such as application seems quite far-fetched, however, as it is fundamentally based on the assumption that conservative dynamics and area-preserving maps realistically describe (or at least approximate) developmental processes, which can surely not be the case for strongly nonequilibrium, dissipative biological systems.

With respect to the important question of potential chaotic processes in development, the following must be noted: A feature of developmental processes is their *stability* and *reproducibility*, in the face of buffetting by persistent environmental perturbations. Embryos are frequently able to regulate in response to even rather drastic disturbances or experimental interventions. Fluctuations may sometimes trigger a self-organizing process, in which case they participate and are utilized positively in developmental mechanisms; but even such 'spontaneous' self-organizing processes, once triggered, are inevitable and stable. The characteristic feature of development is precisely its orderliness and robustness, quite contrary to the sensitivity and instability we would expect from a chaotic process.

Nonlinear science, characterized by irreversibility and systems far from equilibrium, has created a new paradigm for the understanding of the world. Out of this new vision arise two concepts: that of chaos and unpredictability in determinism, and that of self-organization and structure in a complicated system subject to fluctuations. The concept of self-organization provides a powerful framework within which pattern-formation and morphogenesis in development may be analyzed and understood; it appears, however, that chaos is not an appropriate concept for the understanding of developmental processes. We have come full circle from our theoretical deliberations of nonlinearity and complexity of chapter 3, and found not chaos, but *structure and order through self-organization*, in development.

Chapter 7

Conclusion: The Value of Self-Organization Models

We have reached the end of our extensive deliberations on the potential contribution of theoretical approaches, and in particular the self-organization paradigm, in biological development. It remains merely to conclude with a few brief comments in which, rather than attempting to summarize our lengthy discussions, we employ the wide range of insights that has been gained to seek an appreciation of the value of the self-organization approach to development, as presented in this work.

The Wider Context of Self-Organization

Numerous research workers have had little hesitation in applying models based on pure self-organization and random symmetry-breaking to developmental situations, frequently without drawing attention to or clarifying this underlying deep assumption of their models. It has been a concern of this thesis to consider theoretical approaches based on self-organization not only in the light of the necessary genetic, biochemical and biological *experimental* data, but also within their correct *mathematical* and *physical contexts*, which reveal deep connections to mathematical fields such as those of nonlinear dynamical systems and bifurcation theory, and to basic physical and chemical processes such as the Belousov-Zhabotinskii reaction. The aim was thereby to illuminate the underlying *unity* of a wide range of otherwise very diverse theories, not only within the somewhat limited domain of theoretical and mathematical biology, but also within the wider context of the physical and biological sciences. Ultimately, only such a coordinated, holistic interdisciplinary approach, making full use of all the intellectual tools at our disposal, can hold promise for an understanding of the development of complex and functional structures with the property we call Life.

True Symmetry-Breaking in Development?

The conceptual basis of the self-organization paradigm is *symmetry-breaking*, the establishment of novel structure in an initially homogeneous domain disturbed only by random fluctuations. We have seen numerous examples to support the conclusion that models based on this paradigm

have proved their explanatory and, in some cases, even predictive worth in a wide range of developmental situations. In particular, Turing-type reaction-diffusion models and their generalizations play a fundamental role in the study of positional information and chemical prepattern approaches to pattern formation; while more recently, there have been great advances in understanding caused by the application of concepts of self-organization to, *inter alia*, the mechanical and chemical interactions of cells participating individually or in cell sheets in patterning and morphogenetic processes.

In spite of the undoubted worth of many of the models, as we assess the value of the self-organization paradigm it is profitable for us once again to pay somewhat closer attention to the relevance of this fundamental concept to developmental biology. How sound is the premise underlying many models, that there is a pre-existing homogeneity, subject only to random perturbations? There has been some controversy on this point; Harrison [149], for instance, takes issue with the statement of Meinhardt [229, p.39] that "in most biological cases, pattern formation does not involve symmetry breaking ... since the tissue or its environment is asymmetric".

In some cases, there may well be strict symmetry-breaking, although it is probably in general impossible to disprove the existence of cryptic, pre-existing asymmetries; such symmetry-breaking could for instance occur in the initial establishment of axes in the early development of some organisms. In other situations, it does not matter: for integumental patterning, for instance (which has proved to be a very fertile field for applications), there is already considerable structure by the time the pattern is laid down; but there is no need for the patterns to be particularly reproducible, so that the 'stochastic indeterminacy' arising from the amplification of random perturbations by self-organizing processes indeed correlates well with the individuality of the surface markings on animals. But for the vast majority of structures formed in an organism, it is necessary that they be established in a well-defined, *reproducible* location and orientation with respect to the preceding embryonic structures and patterns of which they are a part. Such reproducibility cannot arise from a *purely* random symmetry-breaking process, as self-organizing dynamics tends to display a multiplicity of solutions, with some memory of the initiating fluctuations being retained in the final pattern. To generate the observed stability and reliability of embryonic processes, some initial bias or pre-existing inhomogeneity is always required.

Uniquely Applicable Features of Self-Organization

If symmetry-breaking is far rarer than the wide application of self-organizing mechanisms would indicate, does this concept then have any value for our understanding of development? In the light of the extensive and informative applications we have considered throughout this thesis, the answer must be an unequivocal Yes. For it is not merely the symmetry-breaking properties of self-organizing systems that are pertinent to this study, fundamental and counterintuitive though they might be. The fundamental characteristics — the amplification of weak and essentially structureless inhomogeneities to produce *macroscopic structure*; *long-range correlations*; *memory*, *stability* and *feedback control* — are basic to self-organizing systems and serve to demarcate them from all other mechanisms. *Equilibrium* requirements may account for much of the establishment of structure and change of form that occurs throughout development, whether through diffusion, mechanical deformations, minimization of adhesive energies or any other of the variety of mechanisms available to the embryo; but it is when we augment this range by

including *kinetics*, interactions far from equilibrium, that the truly novel, creative generation of embryonic pattern and form may occur.

Biological development is a coherent, highly coordinated and reproducible spatio-temporal sequence of processes and interactions that combine to produce a structurally and functionally complex organism. Various mechanisms utilized at each of the participating and irreducible hierarchical levels, the genetic, molecular and cellular levels, have been described: reduced to its most basic processes, development occurs as cells divide, grow, deform, change their character, move and die, and communicate with other cells. The molecular and genetic underpinnings of signalling, differentiation, motion and the other cellular activities have been elucidated to a greater or lesser extent, and none of these pose many conceptual problems. But it is in seeking to comprehend the *dynamics* and control of these processes that simple reductionism is no longer sufficient; that one needs to probe beyond simple local interactions and equilibrium properties of the cells, to consider global kinetics and self-organizing processes. It was, indeed, a significant breakthrough when not only the reactions and diffusion of chemicals, but also cellular interactions such as mechanical forces, chemotaxis and cell contact were shown to have self-organizing properties.

The Status of Self-Organization Models

The study of self-organization provides a vivid *paradigm* for our understanding of pattern-forming and morphogenetic processes, a paradigm that directs attention to global correlations and symmetry-breaking, rather than purely local equilibrium interactions. None of the models presented is meant to be taken *literally*: nothing in development is as simple as the two- or three-variable equation systems that were typically presented; these were merely a manifestation of our limited ability to deal analytically and numerically with the mathematical complexities of higher-order systems. The simple models are designed to indicate what is *possible*, to point out the *potential* for self-organization and consequent patterning in a given system — not to attempt detailed accounts of the dynamics of the biochemical interactions of a developing system; the complexity that has been discovered in the *Dictyostelium* gene control systems, leading to highly intricate interactions and models, hints at just how immensely complex and intractable any attempt at realistic modelling must become.

We can furthermore not say whether continuous or discrete models are 'better' for the description of development; both are idealizations of a system which has at root both continuous and discrete aspects. The full potential of cellular automaton models has yet to be explored, but we must say that any formulation which can aid us in gaining *some* increased understanding must be exploited, whatever its mathematical basis. Ultimately, however, the value of a model cannot be deduced from purely theoretical and mathematical considerations, no matter how sound its conceptual foundations or how suggestive its predicted patterns; the true test of any model lies in the continual derivation of predictions that may be compared with experimental observations, a process which may require one to refine or even to discard a model. This is why theories should not be so abstract as to be untestable and unfalsifiable, which is the reason doubt had to be cast on the highly generalized field and catastrophe theory approaches.

Conclusion: Self-Organization in Biological Development

Self-organization in general or in any of its particular manifestations is not 'the answer' to the problem of biological development; no individual mechanism, whether reaction-diffusion processes, gradients or mechanochemical interactions can 'explain' pattern formation, just as embryology cannot be reduced to the study of any *one* of the hierarchical levels of genes, molecules or cells — in particular, the understanding of development is not reducible to the knowledge of the genome, and any putative genetic 'information' or 'program' for development. Each of the multitude and diversity of mechanisms and processes we have encountered, whether having a genetic, equilibrium, self-organizing or any other conceptual foundation, is likely to embody a part of the 'answer', and the complexity of biological systems arises from the intricate interplay of such a wide range of mechanisms. But in broadening the scope of concepts we may profitably employ as we seek to account for the mysteries of our origins, the study of self-organizing processes has certainly played a fundamental and valuable role in our understanding of biological development.

Appendix A

Systems of Reaction-Diffusion Equations

There has been a great deal of interest in the last twenty-five years or so in so-called '**reaction-diffusion**' or 'interaction-diffusion' equations. Such equations describe systems where the components interact and are subject to diffusion in space. They have been profitably applied, for example, in an ecological context, where the 'reaction' terms describe inter- and intraspecific interactions between plant or animal species, and in chemistry and chemical reactor dynamics, as well as biology, where molecular species or enzymes react and diffuse.

The attraction of these equations is in their varied and interesting solution behaviour, which encompasses constant and spatially heterogeneous stationary solutions, as well as oscillatory solutions and travelling waves. In general, the symmetry of the solution may not reflect the symmetry of the problem with its boundary conditions, so that such equations provide a paradigm for *symmetry-breaking* and *self-organization* (see chapter 3). As such, and as possibly the simplest analytical formulation of a system with these self-organizing properties, they have been much studied, both analytically and numerically.

There is a continually increasing number of references on reaction-diffusion equations, which consider their behaviour to varying degrees of rigour and generality; see, for example, [5, 40, 101, 188, 245, 330] and numerous more specialized papers. We are here interested in the pattern-forming, that is, self-organizing capabilities of these equations, so we will just state without proof some of the major pertinent results that have arisen from the study of these equations, before proceeding to an analysis of some conditions for pattern formation and the types of patterns which may be formed. Ultimately, our major concern in this thesis is with applications of self-organization to developmental biology; although such applications will not be treated here (the appendix is essentially purely mathematical), they do motivate the choice of topics that have been included. For this discussions of this appendix, we follow mainly the books by Britton [40] and Murray [251].

Outline of Appendix This appendix is intended as a mathematical introduction to some of the fundamental techniques and results arising in the study of reaction-diffusion equations and similar dynamical systems. As such, it is designed to stand alone, but may be read in

conjunction with section 3.2, where the major results are summarized and the relevance of some of the techniques and findings to the study of self-organization is discussed. The variety of applications to biological development that has been proposed for these equations, beginning with their introduction into the literature by Turing [354], is then presented in chapter 4; and many of the techniques and solution behaviours may also be carried over to the alternative dynamical models for development considered in chapter 5.

The first section of this appendix, A.1, provides a general mathematical formulation of some relevant analytical results about reaction-diffusion equations, and is possibly at the most abstract mathematical level. The linear stability analysis presented in section A.2, and the construction of the first bifurcating solutions after instability in section A.3, are more immediately relevant to applications, although the bifurcation discussions are also placed in a fairly general dynamical systems context. The following section, A.4, is concerned with some general features of the patterns generated by reaction diffusion systems, and the appendix concludes in section A.5 with a brief glimpse at some of the numerical techniques that have been applied to the study of these equations.

A.1 General Analytical Results

We wish in this section to place the study of reaction-diffusion systems into a reasonably rigorous and general context, largely following Britton [40]. For an even more general and abstract treatment, refer for example to Smoller [330].

A.1.1 General System Formulation

We consider, for now, systems of equations defined in arbitrary n-dimensional physical space; and treat the general case of a system of m state variables u_i, so that $\mathbf{u} = (u_1, u_2, \ldots, u_m)^T \in \mathrm{R}^m$. Thus, for example, we may have m chemical species reacting with one another, with concentrations denoted by u_i, in suitable units.

Our equations are defined in a spatio-temporal domain $Q_T \cup S_T$, where (for the study of self-organizing systems) we consider a *finite* bounded spatial domain; so $Q_T = \Omega \times (0,T)$, where $\Omega \subset \mathrm{R}^n$ is bounded. We define $S_0 = \Omega \times \{0\}$, $S_T = \Omega \times \{T\}$, and $\Gamma = \partial\Omega \times (0,T)$, where $\partial\Omega$ is the boundary of Ω in R^n.

With the above definitions, we consider systems of the form

$$\mathbf{u}_t \equiv \frac{\partial \mathbf{u}}{\partial t} = \mathbf{f}(\mathbf{u}) + \mathrm{D}\nabla^2\mathbf{u}, \tag{A.1}$$

or equivalently,

$$N\mathbf{u} = \mathbf{0} \quad \text{in } Q_T \cup S_T; \tag{A.2}$$

$$\text{where} \quad N\mathbf{u} \equiv L\mathbf{u} - \mathbf{f}(\mathbf{u}) = \frac{\partial \mathbf{u}}{\partial t} - \mathrm{D}\nabla^2\mathbf{u} - \mathbf{f}(\mathbf{u}).$$

To complete the specification of the system, we need to include initial conditions

$$\mathbf{u}(\mathbf{x},0) = \mathbf{u}_0(\mathbf{x}) \quad \text{on } S_0, \tag{A.3}$$

and boundary conditions

$$B\mathbf{u} = \mathbf{b}(\mathbf{x}, t) \quad \text{on } \Gamma. \tag{A.4}$$

Here the boundary operator is defined as

$$(B\mathbf{u})_i \equiv c_i(\mathbf{x}, t)u_i(\mathbf{x}, t) + d_i(\mathbf{x}, t)\frac{\partial u_i}{\partial \nu_i}(\mathbf{x}, t), \quad \text{for } i = 1, \ldots, n, \tag{A.5}$$

where $(B\mathbf{u})_i$ is the ith component of the vector $B\mathbf{u}$, $c_i \geq 0$, $d_i \geq 0$, $c_i^2 + d_i^2 > 0$ and $\frac{\partial}{\partial \nu_i}$ denotes an outward derivative.

The above boundary conditions are the most general, or *Robin* boundary conditions. There are some important special cases:

1. $d_i = 0, c_i = 1$, that is boundary conditions

$$\mathbf{u}(\mathbf{x}, t) = \mathbf{b}(\mathbf{x}, t) \quad \text{on } \Gamma;$$

these are *Dirichlet* conditions, with the variable $\mathbf{u}$ specified on the boundary.

2. $c_i = 0, d_i = 1$, or boundary conditions

$$\frac{\partial \mathbf{u}}{\partial \nu}(\mathbf{x}, t) = \nabla \mathbf{u} \cdot \hat{n}(\mathbf{x}, t) = \mathbf{b}(\mathbf{x}, t),$$

where $\hat{n}$ is the unit outward normal; these are *Neumann* conditions, specifying the boundary flux of $\mathbf{u}$. The case of Neumann conditions with $\mathbf{b}(\mathbf{x}, t) = \mathbf{0}$, that is *zero flux boundary conditions*, will be especially important for our analysis. This is because in this case there is no external input, so that any spatial organization or pattern formation must be due to interactions *within* the system, and cannot be influenced by any flows into the system or be the consequence of patterning due to inhomogeneous boundary conditions. It is of course possible to generate structure by the effects of external inputs, such as by imposing asymmetric domain shapes or boundary values; however, such structure does not constitute *novel symmetry-breaking* structure from a previous uniformity, which is ultimately what we seek.

In our original system equations (A.2)–(A.4), D is a constant diagonal diffusion matrix, so that we consider no cross-diffusion for now. The results of the analysis may be extended to include this more general case (see [2]), as well as for D depending on $\mathbf{u}$ or $\mathbf{x}$ (density-dependent or spatially inhomogeneous diffusion), or for the reaction function $\mathbf{f}$ depending on $\mathbf{x}$ or t, or even on $\nabla \mathbf{u}$; for results on such more general reaction systems see the above texts and references cited therein.

A.1.2 Existence and Uniqueness Results

Using the above definitions, and others, a number of general results on existence and uniqueness of solutions, bounds on solutions, comparison theorems and similar analytical results may be obtained; it is, however, always necessary to impose additional conditions, hopefully as unrestrictive as possible, on the equations in order to obtain useful results. To provide a basis for

our further study, and to give a flavour for the types of general results obtainable, we state some of the most relevant results, without proof.

For the discussion in this section, let (P) denote the problem $N\mathbf{u} = 0$, equation (A.2), together with the initial conditions (A.3) and boundary conditions (A.4).

To proceed, we need more definitions:

Definition A.1 (Lipschitz Continuity)

$\mathbf{f}(\mathbf{u})$ is *uniformly Lipschitz continuous* in $\mathbf{u}$ if there exists $K > 0$ such that

$$\|\mathbf{f}(\mathbf{u}_1) - \mathbf{f}(\mathbf{u}_2)\| < K|\mathbf{u}_1 - \mathbf{u}_2| \quad \text{for all} \quad \mathbf{u}_1, \mathbf{u}_2 \in \mathbf{R}^m,$$

where the norm on the right of the inequality is the usual vector norm, and that on the left the L^∞ norm in $\mathbf{R}^m$. □

Definition A.2 (Interior Sphere Property)

Q_T satisfies the *interior sphere property* if for any point $P^* = (\mathbf{x}^*, t^*) \in \Gamma$, there exists a closed ball B such that $B \subset Q_T$, and $B \cap \Gamma = \{P^*\}$; that is, for each boundary point there is a closed ball B contained fully in Q_T which meets the boundary at only that point. □

Then we may obtain a *uniqueness* result:

Theorem A.3 (Uniqueness) *The initial-boundary-value problem (P), where* $\mathbf{f}$ *is uniformly Lipschitz continuous and* Q_T *satisfies the interior sphere property (unless we are concerned with the Dirichlet problem), has at most one solution.*

For a proof, see [40, theorem 5.14].

To prove *existence*, we unfortunately require stricter conditions; in particular, it is often useful to demonstrate the presence of an invariant set.

Definition A.4 (Invariant Set)

A set $\Sigma \subset \mathbf{R}^m$ is an *invariant set* if it satisfies the following condition:

If $\mathbf{u}$ is a solution of

$$N\mathbf{u} = \mathbf{0} \quad \text{in} \quad Q_T \cup S_T,$$

with

$$\mathbf{u}(\mathbf{x}, 0) = \mathbf{u}_0(\mathbf{x}) \in \Sigma \quad \text{for each} \quad (\mathbf{x}, 0) \in S_0,$$

and

$$\begin{aligned} B\mathbf{u}(\mathbf{x}, t) &\in \mathbf{c}(\mathbf{x}, t)\Sigma \\ &\equiv \{(c_1 v_1, c_2 v_2, \ldots, c_m v_m)^T \mid \mathbf{v} \in \Sigma\} \quad \text{for each } (\mathbf{x}, t) \in \Gamma, \end{aligned}$$

then

$$\mathbf{u}(\mathbf{x}, t) \in \Sigma \quad \text{for each point } (\mathbf{x}, t) \in \bar{Q}_T.$$

Thus an invariant set Σ is such that any solution $\mathbf{u}(\mathbf{x},t)$ having all of its boundary and initial values in Σ will satisfy $\mathbf{u} \in \Sigma$ for all $\mathbf{x} \in \Omega$ and for all $t \in [0,T]$. □

This concept of an invariant set is invaluable in obtaining bounds on solutions, and hence obtaining comparison theorems, global existence theorems and similar results — see for example Smoller [330]. By way of iilustration, one set of conditions for global existence (which appears fairly restrictive, as are most available conditions) is given below:

Theorem A.5 (Invariant Rectangles) *Let* $\mathbf{f}$ *be Lipschitz continuous, and let* Q_T *satisfy the interior sphere property (unless we are considering the Dirichlet problem); and let* $\mathbf{a}$ *and* $\mathbf{b}$, $-\infty < \mathbf{a} < \mathbf{b} < \infty$, *be two constant vectors in* $\mathbf{R}^m$ *(where vector inequalities are taken componentwise), such that*

$$\begin{aligned} f_i(\mathbf{v}) &\geq 0 \quad \text{for } v_i = a_i, \quad \mathbf{a} \leq \mathbf{v} \leq \mathbf{b} \\ f_i(\mathbf{v}) &\leq 0 \quad \text{for } v_i = b_i, \quad \mathbf{a} \leq \mathbf{v} \leq \mathbf{b}. \end{aligned}$$

Then $\Sigma \equiv \{\mathbf{v} | \mathbf{a} \leq \mathbf{v} \leq \mathbf{b}\} \subset \mathbf{R}^m$ *is an invariant set for the initial-boundary-value problem, (P), that is, (A.2) with (A.3) and (A.4).*

For a proof, see Britton [40, theorem 5.17].

Global existence of solutions follows if such an invariant set exists:

Theorem A.6 (Global Existence) *Let the conditions of theorem (A.5) hold. Then the initial-boundary-value problem (P) with initial conditions* $\mathbf{u}_0 \in \epsilon\Sigma$ *(where* ϵ *arises in the proof of theorem (A.5) — see [40, theorem 5.17]) and boundary conditions* $B\mathbf{u} \in \mathbf{c}\Sigma$ *has a global solution, which is unique, by theorem (A.3).*

For a proof, see Britton [40, theorem 5.18].

As indicated above, in general the existence of solutions is difficult to show, whereas uniqueness follows from fairly unrestrictive conditions. But what we are really interested in is not so much the uniqueness of a solution for fixed initial conditions, as in the effect of small fluctuations in the initial conditions — will these lead to a solution that is essentially the same as before, or to a totally different one? For if the statistical random fluctuations in initial conditions, which are always present in any physical situation, lead to qualitative changes in the final solution, with novel structure of symmetry properties, then we may say that the system has *self-organized* — we obtain *order out of fluctuations*.

A.1.3 Stability and Linearization

Thus we are led to consider the concept of *stability*; informally, we consider a solution to be stable if small changes in the initial conditions die out, and unstable if they grow to give rise to a final solution significantly different from the solution whose stability we are considering.

(In our considerations of stability, we work in the L^∞ norm, given by

$$\|\mathbf{h}(\cdot)\|_{L^\infty} \equiv \sup_{\mathbf{x} \in \Omega} |\mathbf{h}(\mathbf{x})|,$$

where $|\cdot|$ is a vector norm (for example the usual Euclidean norm) of vector $\mathbf{h}$. We denote, in the following, this L^∞ norm simply by $\|\cdot\|$. Note that other norms could be used. We are here also interested in the *long time behaviour* of solutions, so that in contrast to the above considerations, we treat solutions defined for arbitrarily large times from now on.)

Thus stability of a solution is defined by:

Definition A.7 (Stability)

Consider the solution $\mathbf{u} = \phi(\mathbf{x},t)$ of $N\mathbf{u} = \mathbf{0}$ in $\Omega \times (0,\infty)$, satisfying $\phi(\mathbf{x},t) = \phi_0(\mathbf{x})$ for $\mathbf{x} \in \Omega$, and $B\phi = \mathbf{b}$ on $\partial\Omega \times (0,\infty)$. Let $\mathbf{u} = \psi(\mathbf{x},t)$ be some other solution satisfying $N\psi = \mathbf{0}$ in $\Omega \times (0,T)$ for some $T > 0$, and $B\psi = \mathbf{b}$ on $\partial\Omega \times (0,T)$. Then ϕ is a *stable solution* of the initial-boundary-value problem (P) if, given any $\epsilon > 0$, there exists δ such that whenever $\psi(\mathbf{x},0) = \psi_0(\mathbf{x})$ satisfies

$$\|\phi_0(\cdot) - \psi_0(\cdot)\| < \delta$$

then (i) ψ may be continued to be a solution of $N\psi = \mathbf{0}$ in $\Omega \times (0,\infty)$ with $B\psi = \mathbf{b}$ on $\partial\Omega \times (0,\infty)$, and (ii)

$$\|\phi(\cdot,t) - \psi(\cdot,t)\| < \epsilon$$

for all $t > 0$; ϕ is *asymptotically stable* if, in addition, δ can be chosen so that

$$\|\phi(\cdot,t) - \psi(\cdot,t)\| \to 0 \quad \text{as} \quad t \to \infty.$$

A solution is *unstable* if it is not stable. □

In the light of the above definition, we consider the stability of stationary (time-independent) solutions of reaction-diffusion equations; this may be analysed by considering the eigenvalues of the linearized system. This stability analysis is a fundamental aspect of our study of reaction-diffusion equations, as it will enable us to deduce conditions for which spontaneous pattern formation is possible.

We restrict ourselves to zero flux boundary conditions, and note that in our quest for stationary solutions, we may consider the long-time behaviour, as $T \to \infty$, so that the system can asymptotically settle down to a stationary solution; hence we consider a global solution, defined for all $t \geq 0$, for the problem

$$\mathbf{u}_t = \mathbf{f}(\mathbf{u}) + D\nabla^2\mathbf{u} \quad \text{in} \quad \Omega \times (0,\infty), \tag{A.6}$$

where Ω is a bounded domain, and D a diagonal matrix, with zero-flux boundary conditions

$$\frac{\partial \mathbf{u}}{\partial \nu}(\mathbf{x},t) = \mathbf{0} \quad \text{on} \quad \partial\Omega \times (0,\infty), \tag{A.7}$$

and initial conditions

$$\mathbf{u}(\mathbf{x},0) = \mathbf{u}_0(\mathbf{x}) \quad \text{on} \quad \Omega \times \{0\}. \tag{A.8}$$

We assume that $\mathbf{u} = \mathbf{0}$ is a steady state of the above equations (any other steady state $\mathbf{u} = \mathbf{u}^*$ may be brought to $\mathbf{0}$ by a simple linear transformation $\mathbf{U} = \mathbf{u} - \mathbf{u}^*$) and linearize about $\mathbf{0}$.

Thus we decompose $\mathbf{f}(\mathbf{u})$ into a linear and nonlinear part by

$$\mathbf{f}(\mathbf{u}) = A\mathbf{u} + \mathbf{N}(\mathbf{u}), \tag{A.9}$$

where A is a matrix and $\mathbf{N}(\mathbf{u}) = o(\mathbf{u})$ as $\mathbf{u} \to \mathbf{0}$, that is

$$|\mathbf{N}(\mathbf{u})|/|\mathbf{u}| \to 0 \quad \text{as} \quad \mathbf{u} \to \mathbf{0}.$$

The linearized system is defined to be

$$\mathbf{u}_t = A\mathbf{u} + \mathrm{D}\nabla^2\mathbf{u}. \tag{A.10}$$

With the boundary and initial conditions as given, the problem (A.10) may be solved by the method of *eigenfunction expansions*:

We denote the eigenvalues of $-\nabla^2$ in Ω with homogeneous Neumann boundary conditions by μ_n, and the corresponding normalized eigenfunctions by ϕ_n, noting that for this Helmholtz problem, the eigenfunctions are orthogonal and the eigenvalues all non-negative; that is,

$$-\nabla^2\phi_n = \mu_n\phi_n, \tag{A.11}$$

where

$$0 = \mu_0 < \mu_1 \leq \mu_2 \leq \cdots$$

(For such positive eigenvalues, we may write $\mu_n = k_n^2$, where k_n is the wave number, and $1/k_n$ is proportional to the wavelength λ. For example, for a one-dimensional domain $\Omega = (0, a)$, the eigenfunctions are $\phi_n \propto \cos(n\pi x/a)$, where n is an integer; the eigenfunctions satisfy zero flux conditions at $x = 0$ and $x = a$, and the wave number in this case is $k_n = n\pi/a$, with a corresponding wavelength $\lambda_n = 2\pi/k_n = 2a/n$. We can thus already see that for such finite domains, there is a discrete set of possible wavenumbers. This is closely related to the types of patterns that can be formed, and will be discussed in greater detail later.)

We define the vector coefficient $\mathbf{u}_{0n}$ of the eigenfunction ϕ_n in the expansion of the initial condition $\mathbf{u}_0(\mathbf{x})$ in terms of the orthogonal eigenfunctions by

$$\mathbf{u}_{0n} = \int_\Omega \mathbf{u}_0(\mathbf{x})\phi_n(\mathbf{x})d\mathbf{x}. \tag{A.12}$$

Now let $A_n = A - \mu_n\mathrm{D}$, and let $\exp(A_n t)$ be the matrix solution of the differential equation

$$\frac{d}{dt}[\exp(A_n t)] = A_n \exp(A_n t), \quad \text{with initial condition } \exp(A_n 0) = I. \tag{A.13}$$

Then the solution of the linear system (A.10) with zero flux boundary conditions may be written in the form

$$\mathbf{u}(\mathbf{x}, t) = \sum_{n=0}^{\infty} \phi_n(\mathbf{x}) \exp(A_n t)\mathbf{u}_{0n}, \tag{A.14}$$

as may be confirmed by direct substitution.

It follows after some analysis that the stability of the zero solution depends on the eigenvalues of the matrices A_n, and we state the theorem, again without proof:

Theorem A.8 *1. The zero solution of the linear system (A.10) with zero flux boundary conditions is globally asymptotically stable if for each non-negative integer n the eigenvalues of $A_n = A - \mu_n \mathrm{D}$ have negative real parts. Furthermore there exist positive constants K and σ such that for any $t > 0$*

$$\|\mathbf{u}(\cdot,t)\| \leq Ke^{-\sigma t}\|\mathbf{u}_0(\cdot)\|.$$

2. The zero solution is stable if for each non-negative integer n the eigenvalues of A_n have non-positive real parts and those with zero real parts have simple elementary divisors.

3. The zero solution is unstable if for some n there exists an eigenvalue of A_n with either positive real part or zero real part with a non-simple elementary divisor.

See Casten and Holland [49] for proof.

It follows immediately that if D is a scalar matrix, $\mathrm{D} = dI$, then diffusion cannot destabilize the zero solution: if ν is an eigenvalue of A, then $\nu - \mu_n d$ is the corresponding eigenvalue of A_n; $\mu_n \geq 0$ and $d \geq 0$, so that $\nu - \mu_n d \leq \nu$. As scalar diffusion can only decrease, not increase, the eigenvalues, it can hence only stabilize, not destabilize the system. However, if the diffusion matrix is not scalar, then the above brief analysis does not hold, and diffusion *can* destabilize an otherwise stable steady state, as we will show in detail later.

The last important general question is: how does the stability behaviour of the nonlinear system follow from that of the linear system? One result concerning this difficult question is that asymptotic stability carries over:

Theorem A.9 (Linearized Stability) *The zero solution of the general reaction-diffusion system, (A.6) with boundary conditions (A.7), is asymptotically stable if the zero solution of the corresponding linear system, (A.10) with (A.7) is.*

For a proof, see [49] or [40, theorem 5.61].

A.1.4 Some Nonexistence Results for Reaction-Diffusion Spatial Patterns

We will proceed in section A.2 with a general linear stability analysis to indicate some of the conditions for which 'diffusion-driven' instability may occur, as this is crucial in the context of spatial patterning and self-organization. Before proceeding, however, we state two results indicating some general restrictions on the conditions for which patterning may occur. The results are given briefly and informally, and are thus not presented as theorems.

1. *A scalar reaction-diffusion equation (only one state variable) in one space dimension with zero flux boundary conditions cannot sustain a spatial pattern.*

This result may be stated more rigorously: in the problem

$$u_t = f(u) + u_{xx}, \quad x \in (0,1) \text{ (normalized domain)}, \; t > 0, \tag{A.15}$$

$$\text{with} \quad u_x(0,t) = u_x(1,t) = 0, \quad t > 0;$$

if $U(x)$ is a steady state solution (that is, it satisfies $U'' + f(U) = 0, \quad U'(0) = U'(1) = 0$), which is spatially nonuniform (that is $U'(x)$ is not identically zero) then $U(x)$ is unstable (see for example [251, pp.424–426] for proof).

Thus, for instance, at least two interacting species of chemicals are needed to produce pattern formation in one spatial dimension. The result does not carry over fully to higher dimensions; Casten and Holland [50] have demonstrated the nonexistence of stable nonconstant stationary solutions of the Neumann problem for arbitrary functions f in *convex* domains, but in non-convex domains such solutions have been shown to be possible [225].

2. We have seen that spatial patterning is not possible for scalar diffusion, but, as we shall see later, different diffusion coefficients may permit the formation of spatial inhomogeneity. This fundamental result, first due to Turing [354], seems counterintuitive, as one tends to regard diffusion as a mechanism for smoothing out spatial disturbances. Thus it at least appears reasonable that *if the diffusion coefficients are large enough, diffusion can damp out all spatial variation*, and for general multi-species systems patterning can be destroyed.

More precisely, we can define an 'energy' $E(t)$ by

$$E(t) = \tfrac{1}{2} \int_\Omega \|\nabla \mathbf{u}\|^2 d\mathbf{x}, \tag{A.16}$$

where in this case the norm $\|\cdot\|$ is the usual matrix norm given by

$$\|\nabla \mathbf{u}\|^2 = \sum_{i=1}^{n} |\nabla u_i|^2 = < \nabla \mathbf{u}, \nabla \mathbf{u} >;$$

clearly $E(t)$ is a measure of the spatial variations in the system. Let d be the smallest eigenvalue of the matrix D; define

$$m = \max_{\mathbf{u}} \|\nabla_{\mathbf{u}} \mathbf{f}(\mathbf{u})\| \tag{A.17}$$

(where $\mathbf{u}$ takes on all possible solution values and $\nabla_{\mathbf{u}}$ is the gradient operator with respect to the components of $\mathbf{u}$); and let μ_1 be the least positive eigenvalue of $-\nabla^2$ in Ω, as defined above, in section A.1.3.

Then it can be shown that

$$\frac{dE}{dt} \leq (m - 2\mu_1 d)E,$$

$$\text{which implies} \quad \lim_{t \to \infty} E(t) = 0 \quad \text{if} \quad m < 2\mu_1 d. \tag{A.18}$$

Thus $\nabla \mathbf{u} \to \mathbf{0}$ and all spatial patterns are damped out as $t \to \infty$, if the smallest diffusion coefficient d is large enough (see [251, pp.426–429] or [40, section 5.7] for proof).

This result on diffusive damping may be interpreted as follows [251]: One can consider $1/m$ as a measure of the shortest kinetic relaxation time of the mechanism, and $1/2\mu_1 d$ as an estimate of the longest diffusion time. The above result, $1/m > 1/2\mu_1 d$, then implies that if the shortest relaxation time is longer than the longest diffusion time, all spatial patterning which might be produced will be damped out by the rapidly acting diffusion as $t \to \infty$, and the system mechanism will be governed solely by the kinetics.

We have considered two general situations within which spatial patterning cannot occur; let us thus proceed to consider in more detail the situation in which spatial inhomogeneities *may* be produced in the presence of diffusion.

A.2 Linear Stability Analysis

We have considered general conditions for stability of reaction-diffusion systems, together with some existence and uniqueness results. Many more analogous results may be obtained — see any of the references mentioned — but in order to obtain more specific knowledge about solution behaviour and stability, and in particular about pattern-forming capability, we need to restrict ourselves to a more specific situation.

We have indicated above that pattern formation is very limited for scalar reaction-diffusion equations, so that we proceed to study the case of two interacting species, $m = 2$, in more detail; $m \geq 3$ is much harder to analyse, and for the two-variable situation we have the benefit of the powerful geometrical techniques of phase-plane analysis available to us. Furthermore, both for mathematical simplicity and to ensure that we have self-organization as opposed to externally imposed spatial structure, we restrict ourselves to zero flux boundary conditions, as discussed above.

In this context our mathematical problem (A.1)–(A.4) may be conveniently formulated as (setting the state vector $\mathbf{u} = (u, v)^T$)

$$u_t = \gamma f(u,v) + \nabla^2 u \tag{A.19}$$

$$v_t = \gamma g(u,v) + d\nabla^2 v \tag{A.20}$$

with

$$\hat{n} \cdot \nabla \begin{pmatrix} u \\ v \end{pmatrix} = 0 \quad \text{on} \quad \partial\Omega, \tag{A.21}$$

and

$$u(\mathbf{x}, 0) = u_0(\mathbf{x}), \quad v(\mathbf{x}, 0) = v_0(\mathbf{x}) \ \text{ given.} \tag{A.22}$$

This form of the equations [251] may be obtained from completely general reaction functions $\mathbf{f}(\mathbf{u})$ and diffusion matrix D by an appropriate *nondimensionalization*. This technique is demonstrated by means of a specific example, the 'Brusselator', in section 3.2.3; see also appendix A.4 for a further motivation of its usefulness. Nondimensionalization is valuable in that it allows one to analyse the system independent of scales and units, and hence enables one to compare different systems; it brings out clearly the fact, to be elaborated on later, that it is in general *combinations* of parameters that have pattern forming properties.

For a given system, different nondimensionalizations are possible in general, which may bring out different properties; in the above general scheme, γ is a scale parameter which will allow us to assess the effect of domain size on pattern formation, and d is the ratio of the diffusion coefficients of the two species. The above scheme has been much studied by Murray (see for example [251]), and enables us to derive *necessary and sufficient conditions* for diffusion-driven instability. The techniques to be discussed in some detail here are quite standard, but important enough to be repeated; this method of linear stability analysis has been repeated at numerous points throughout the literature (for one of the earliest general discussions for reaction-diffusion equations, see [319]).

A.2.1 Stability to Homogeneous Disturbances

In terms of our interest in self-organization, we are most interested in the situation where a homogenous steady state is stable to homogeneous disturbances, but unstable to spatially dependent perturbations, as the spatial dependence introduced by diffusion then produces the desired pattern-forming effects.

The homogeneous steady state (u_0, v_0) is a positive solution of

$$f(u,v) = 0, \quad g(u,v) = 0. \tag{A.23}$$

We linearize about this steady state by considering small perturbations

$$\mathbf{w} = \begin{pmatrix} u - u_0 \\ v - v_0 \end{pmatrix}. \tag{A.24}$$

Then the general two-variable reaction-diffusion system becomes, in a linearized form (that is, $|\mathbf{w}|$ small),

$$\mathbf{w}_t = \gamma A\mathbf{w} + D\nabla^2\mathbf{w}, \tag{A.25}$$

where A is the Jacobian, or stability, matrix (denoted community matrix in ecology)

$$A = \begin{pmatrix} f_u & f_v \\ g_u & g_v \end{pmatrix}_{u_0,v_0}$$

with all partial derivatives in this section evaluated at the steady state, and

$$D = \begin{pmatrix} 1 & 0 \\ 0 & d \end{pmatrix}.$$

We consider first spatially independent perturbations, and derive the condition for the stability of the steady state to such perturbations.

With no spatial variations (A.25) becomes

$$\mathbf{w}_t = \gamma A\mathbf{w}. \tag{A.26}$$

We look for solutions of the form $\mathbf{w} \propto e^{\sigma t}$; then by substitution we see that σ satisfies the eigenvalue problem $\gamma A\mathbf{w} = \sigma\mathbf{w}$, so that for a nontrivial solution the eigenvalues σ are determined as the solution of

$$\det(\gamma A - \sigma I) = \begin{vmatrix} \gamma f_u - \sigma & \gamma f_v \\ \gamma g_u & \gamma g_v - \sigma \end{vmatrix} = 0, \tag{A.27}$$

which implies

$$\sigma_1, \sigma_2 = \tfrac{1}{2}\gamma\left[(f_u + g_v) \pm \left\{(f_u + g_v)^2 - 4(f_u g_v - f_v g_u)\right\}^{1/2}\right] \tag{A.28}$$

Linear stability, that is Re $\sigma < 0$ for both solutions, is thus guaranteed if

$$\operatorname{tr} A = f_u + g_v < 0; \tag{A.29}$$

$$\det A \equiv |A| = f_u g_v - f_v g_u > 0. \tag{A.30}$$

As u_0, v_0 and the partial derivatives are functions of the kinetic parameters, these inequalities impose certain *constraints* on the parameters.

A.2.2 Instability to Spatially Varying Perturbations

We now consider the full linearized reaction-diffusion system (A.25), and note as above that the solution can be written in terms of the eigenfunctions $\phi_n(\mathbf{x})$ and corresponding positive eigenvalues μ_n of $-\nabla^2$ in Ω, with zero flux boundary conditions, as in equation (A.14) (see section A.1.3).

Thus we may use the above theorem (A.8), together with theorem (A.9) which permits the extension of the results to nonlinear reaction-diffusion equations, to deduce that the stability of the zero steady state of (A.25) depends on the eigenvalues σ of the matrices $A_n = \gamma A - \mu_n D$, which determines the eigenvalues as the roots of the characteristic equation

$$\det(\gamma A - \mu_n D - \sigma I) = 0. \tag{A.31}$$

As we have noted above, $\mu_n \geq 0$, so that it is useful to write $\mu_n = k_n^2$, where k_n is the wavenumber, proportional to the inverse wavelength $1/\lambda_n$ (we consider for now just a general wave number k).

Note that a similar expression for the eigenvalues σ is obtained by going not via the matrix exponential, but by the analogous method of assuming a solution of the form

$$\mathbf{w}(\mathbf{x}, t) = \sum_{n=0}^{\infty} \mathbf{u}_{0n} e^{\sigma t} \phi_n(\mathbf{x}) \tag{A.32}$$

(see for example [251]). Also, for situations where $-\nabla^2$ has a continuous spectrum as opposed to discrete eigenvalues, such as for reaction-diffusion systems on an unbounded domain, we must consider a more general spectral problem [40].

We evaluate the above determinant (A.31) with A and D as given above, to find the eigenvalues σ, as functions of the squared wavenumber k^2, as the roots of

$$\sigma^2 + \sigma(k^2(1+d) - \gamma(f_u + g_v)) + h(k^2) = 0, \tag{A.33}$$

$$\text{where} \quad h(k^2) = dk^4 - \gamma(df_u + g_v)k^2 + \gamma^2(f_u g_v - f_v g_u). \tag{A.34}$$

By the theorems quoted above, the steady state (u_0, v_0) of the reaction-diffusion system is linearly stable, and hence asymptotically stable, if both solutions σ have $\text{Re}\ \sigma < 0$. From the theory of quadratic equations (or the Routh-Hurwitz conditions for $n = 2$) this holds if the constant in (A.33)

$$h(k^2) > 0, \tag{A.35}$$

and the coefficient of σ is positive, that is

$$k^2(1+d) - \gamma(f_u + g_v) > 0. \tag{A.36}$$

Instability occurs if either of these inequalities does not hold.

We have already required the steady state to be stable to spatially independent disturbances, that is those with $k = 0$, giving the above conditions (A.29)–(A.30); we require instability for spatially dependent perturbations, $k \neq 0$. But from the conditions for $k = 0$, $f_u + g_v < 0$, so that the coefficient of σ,

$$k^2(1+d) - \gamma(f_u + g_v) > 0 \quad \text{always.} \tag{A.37}$$

Hence the only possibility for instability, ie. Re $\sigma > 0$, is if for some k,

$$h(k^2) = dk^4 - \gamma(df_u + g_v)k^2 + \gamma|A| < 0. \tag{A.38}$$

Since $|A| > 0$ from the conditions for $k = 0$, $h(k^2)$ can only become negative if

$$df_u + g_v > 0. \tag{A.39}$$

This combined with $f_u + g_v < 0$ indicates that $d \neq 1$, that is the diffusion coefficients must be unequal. We have already obtained this requirement of nonscalar diffusion more generally in a corollary to theorem (A.8). Also, this indicates that f_u and g_v must have opposite signs. Without loss of generality we can label our reacting state variables u and v so that $f_u > 0$, $g_v < 0$; then the condition (A.39) requires $d > 1$ (this may be interpreted physically as the requirement that an inhibiting chemical substance diffuses faster than the activator — see section 3.2.2 [87, 114]).

The last inequality (A.39) is necessary but not sufficient for Re $\sigma > 0$; we require more generally that the minimum of $h(k^2)$ must be negative. By differentiating (A.38) with respect to k^2, we find that

$$h_{\min} = \gamma^2 \left[|A| - \frac{(df_u + g_v)^2}{4d} \right] \tag{A.40}$$

at

$$k^2 = k_m^2 = \gamma \frac{df_u + g_v}{2d}. \tag{A.41}$$

Thus the condition that $h_{\min} < 0$ is

$$\frac{(df_u + g_v)^2}{4d} > |A|. \tag{A.42}$$

This defines the last condition for instability; we thus have four inequalities, (A.29), (A.30), (A.39) and (A.42), which must be satisfied for a *stable homogeneous steady state to be driven unstable by spatial perturbations in the presence of diffusion*, that is for *diffusion-driven*, or *Turing*, instability to occur.

When $h_{\min} < 0$, instability can occur for those values of k for which $h(k^2) < 0$, that is for $k_1 < k < k_2$, where k_1 and k_2 are the roots of $h(k^2) = 0$. There is thus only a finite range of wave numbers, and hence of wavelengths, for which diffusion-driven instability will occur.

Clearly there is a critical point where $h_{\min} = 0$ — this defines a *bifurcation point* (see the next section A.3 for a discussion of bifurcations) at which

$$|A| = \frac{(df_u + g_v)^2}{4d}. \tag{A.43}$$

Thus this point defines a critical diffusion coefficient ratio, $d_c (> 1)$, given by the appropriate root of

$$d_c^2 f_u^2 + 2(2f_v g_u - f_u g_v)d_c + g_v^2 = 0, \tag{A.44}$$

with a corresponding critical wave number k_c, given by

$$k_c^2 = \gamma \frac{d_c f_u + g_v}{2d_c} = \gamma \left[\frac{|A|}{d_c} \right]^{1/2} = \gamma \left(\frac{f_u g_v - f_v g_u}{d_c} \right)^{1/2}. \tag{A.45}$$

A.3 Bifurcation Analysis

Our previous analysis has indicated the existence of conditions for which an equilibrium solution becomes unstable; in particular we have sought, and found, situations for which a homogeneous steady state of a reaction-diffusion system is driven unstable with respect to spatially inhomogeneous disturbances by diffusion. We noted also that if we kept all parameters except one constant, there was a critical value of the parameter of interest for which this instability set in.

Thus we are driven out of the realm of a featureless, patternless equilibrium, into a new domain. Linear stability analysis can indicate the presence of this transition, and aid us in locating it, but it gives no direct indication of the behaviour of the solutions beyond the point of instability.

So what happens after the homogeneous steady state becomes unstable? A linear reaction-diffusion system has fairly uninteresting behaviour, as linear stability analysis gives global stability, so that an unstable solution diverges exponentially to infinity. But in a nonlinear system, the nonlinear terms ignored in the linear stability analysis may provide damping, so that a solution other that the homogeneous steady state may become a stable stationary solution, giving rise to a new equilibrium domain.

One of the most powerful techniques for obtaining qualitative and some quantitative results about the behaviour of dynamical systems, in particular at instabilities, under conditions where symmetry-breaking and self-organization occur, is *bifurcation theory*.

A.3.1 Introduction to Bifurcations

Bifurcation theory in general is the study of equilibrium solutions of evolution equations of the form

$$\mathbf{u}_t = \mathrm{F}(\lambda, \mathbf{u}), \tag{A.46}$$

where $\mathbf{u}$ is in some Hilbert space H (or more generally a Banach space B), $\mathrm{F}(\lambda, \cdot)$ is some operator, and λ is a parameter which we take for now to be scalar for simplicity (for more general treatments of bifurcations in terms of the unfoldings of vector fields we must consider λ to be vector-valued — see also the discussion of catastrophe theory in section 6.1).

In the context of reaction-diffusion systems, the evolution equations can generally be written as

$$\mathbf{u}_t = \mathbf{f}(\lambda, \mathbf{u}) + D\nabla^2\mathbf{u}, \tag{A.47}$$

where $\mathbf{f}(\lambda, \mathbf{u})$ represents the nonlinear reaction terms. Out of the various parameters present in $\mathbf{f}$, we have chosen one as the bifurcation parameter of interest (after nondimensionalization, this λ will usually be a combination of various of the dimensional kinetic parameters of the system). Also, for the following general discussion we will take D to be independent of λ, which appears to exclude our previous analysis (where we considered d as the bifurcation parameter) but this restriction on D is only for convenience now; it is not necessary [40]. We further assume that $\mathbf{f}$ is not explicitly dependent on $\mathbf{x}$ or t.

The Hilbert space H will usually be the completion under the norm, derived from a suitable inner product $< \cdot, \cdot >$, of the space of functions $\mathbf{u}(\mathbf{x}, t)$ (defined on a domain $\Omega \times (-\infty, \infty)$,

where $\Omega \subset \mathbf{R}^n$), which are sufficiently smooth for the relevant derivatives to be continuous, and which are such that the relevant boundary conditions (or conditions at infinity) are satisfied.

The equilibrium solutions under consideration in bifurcation theory may be steady or periodic solutions, or have a more complex time dependence (sub-harmonic solutions, or asymptotically quasi-periodic solutions, which arise by bifurcation from periodic solutions). Bifurcation theory deals mainly with the manner in which the system passes from one equilibrium solution to another as the parameter λ is varied; and, as unstable equilibria are never observed under the conditions of small random fluctuations always present in real situations, we need to consider the stability as well as the existence and structure of the bifurcating solutions.

The Scope of our Discussion As before, the present discussion will make no attempt at comprehensiveness, but will serve just to indicate some of the techniques that are useful in the study of systems of nonlinear reaction-diffusion equations, or are more generally applicable to some bifurcation situations in dynamical systems. The results are not motivated, as would usually be done, by first giving one-dimensional results and then extending them; apart from a brief introduction to bifurcations by means of a simple example, we proceed directly to the general (infinite-dimensional) case.

For a fairly elementary introduction to bifurcations, with specific reference to applications to self-organization in dynamical systems, refer to [10, 277]. The book by Nicolis and Prigogine [276] gives a good introductory treatment, with specific reference to the Brusselator model discussed in section 3.2.3; for a more general introduction, see Britton [40], whose treatment we largely follow. A more detailed discussion of bifurcation theory is given for example by Guckenheimer and Holmes [138].

Heuristically, the concept of bifurcation refers to the situation where a system that had been in a stable equilibrium situation, suddenly 'finds itself with a choice' — the previous equilibrium has become unstable as the bifurcation parameter λ attains the critical value λ_c, and a new equilibrium has to be sought.

A Simple Example: the Pitchfork Bifurcation

A simple but distinctly nontrivial example (if considered in all its ramifications) in one variable is

$$\dot{x} \equiv \frac{dx}{dt} = \lambda x - x^3. \tag{A.48}$$

For $\lambda < 0$, $x = 0$ is the only equilibrium solution (steady state), and a simple analysis shows it to be stable — the linearized system is $\dot{x} = \lambda x$ with eigenvalue λ. However, for $\lambda > 0$, $x = 0$ becomes unstable, and simultaneously two new, stable steady states, $x = \pm\sqrt{\lambda}$, appear; thus $\lambda = \lambda_c = 0$ is the bifurcation point, beyond which multiple solutions are possible. As λ increases through 0, the system is forced to choose between the two possible stable steady states $\pm\sqrt{\lambda}$, and the later behaviour of the system will be determined by whether at the bifurcation point, fluctuations from the equilibrium were positive or negative. Nothing in the equations (or, in a physical system, in the experimental setup) enables one to decide beforehand which state will be chosen; the trajectory beyond bifurcation is totally dependent on the random fluctuations present at the bifurcation point, where eventually one fluctuation will predominate

and drive the system away from its unstable equilibrium to a new stationary state. Whereas the original equilibrium was symmetric about $x = 0$, thus maintaining the same symmetry as the equations (or the physical system), after bifurcation this symmetry is lost, as the solution becomes definitely either positive or negative, so that we have *symmetry-breaking* [277].

The basic equilibrium properties of this simple differential equation may be appreciated readily by plotting the equilibrium solutions for the state variable x against the parameter λ, to yield the bifurcation diagram for the *pitchfork bifurcation*, as in figure A.1(a).

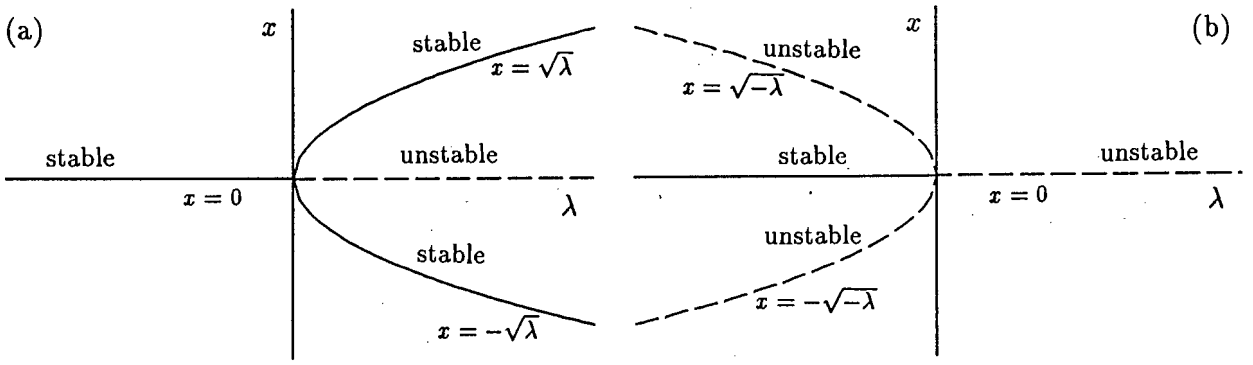

Figure A.1: Pitchfork bifurcation diagram, showing how the equilibrium solutions for the state variable x are affected when the control, or bifurcation, parameter λ varies: (a) Supercritical bifurcation, equation (A.48); (b) Subcritical bifurcation, equation (A.54).

Implications concerning Self-Organization The Bénard experiment described in section 3.1.2 demonstrates this concept of bifurcation clearly: with the temperature gradient as bifurcation parameter, there is a critical ΔT for which the state of stationary convection becomes unstable and the system passes to the new regime of convective rolls, with a concomitant loss of translational symmetry. Nothing in the experiment indicates whether the direction of rotation in the first cell will be clockwise or anticlockwise; that choice, with its macroscopic ramifications, is left to microscopic variability, to chance. Thus we perceive the intricate interplay between stochasticity and determinism, between chance and necessity, which was noted in section 3.1 as a fundamental 'ingredient' of self-organization [274].

The eventual situation is a non-equivalence, or intrinsic differentiation between parts of a system; the novel features which bifurcation has introduced in the departure from the originally featureless steady state may be considered the 'beginnings of information' — indeed, the innovation and diversification between initially equivalent parts of a system introduced through bifurcation, as it endows the system with new and novel solutions, may be regarded as a prerequisite for any information to exist.

We have discussed the simple and intuitively obvious example of the pitchfork bifurcation as it displays many of the features of bifurcation, which are also pertinent to reaction-diffusion systems: in particular, we see the possibility of multiple stable solutions after the bifurcation point (which does not contradict the uniqueness result quoted above, theorem (A.3), as the solution 'chosen' depends on the, possibly random, initial conditions). Also, the final state depends on the fluctuations from a steady state of high symmetry which was initially stable, but

is driven unstable due to variations in some parameter, in reaction-diffusion equations connected to the effect of diffusion; so that symmetry-breaking occurs. In reaction-diffusion equations, it is the spontaneous loss of spatial homogeneity (driven only by internal statistical fluctuations) and the concomitant generation of spatial pattern and structure which interests us most.

Symmetry-Breaking and Bifurcation in Reaction-Diffusion Systems

A general discussion of bifurcation in reaction-diffusion equations would require a rigorous definition of the relevant function spaces involved, and a detailed derivation of the solutions after instability; a derivation which is best motivated beforehand by the study of bifurcations in one dimension and in a finite number of dimensions, before proceeding to the infinite-dimensional case of reaction-diffusion systems (where the stationary solution $\mathbf{u}$ we seek depends on $\mathbf{x}$ as well as on a finite number of parameters, so that $\mathbf{u} \in H$, a Hilbert space). As this appendix is intended as an introduction to the pertinent bifurcation phenomena, rather than a detailed derivation which will provide little new *understanding* of bifurcations in the context of self-organization, we skip the preliminaries and proceed immediately to state the relevant results, in the light of the required definitions; for a more rigorous discussion, from which we abstract the comments below, see especially [40, ch.6].

As indicated above, in bifurcation theory we are interested in the stationary solutions of the system

$$\mathbf{u}_t = \mathbf{F}(\lambda, \mathbf{u}) = \mathbf{f}(\lambda, \mathbf{u}) + D\nabla^2\mathbf{u}, \quad \mathbf{f}(\lambda, \mathbf{0}) = \mathbf{0} \tag{A.49}$$

where λ is a parameter, and $\mathbf{u} = (u_1, u_2, \ldots, u_m)^T \in B$, where B is in general some Banach space; in the case of stationary solutions of reaction-diffusion systems, $B = C^2(\Omega, \mathbf{R}^m)$, where $\Omega \subset \mathbf{R}^n$.

Bifurcation occurs when the steady solution (which we can take to be $\mathbf{u} = \mathbf{0}$, without loss of generality) of (A.49) becomes unstable. Thus, as indicated in our previous general discussions, we linearize (A.49) about the equilibrium solution ($\mathbf{u} = \mathbf{0}$), as before, to give

$$\begin{aligned} \mathbf{u}_t &\approx L(\lambda)\mathbf{u} \\ &= M(\lambda)\mathbf{u} + D\nabla^2\mathbf{u}, \end{aligned} \tag{A.50}$$

where $L(\lambda) \equiv M(\lambda) + D\nabla^2$ is the linear operator obtained by linearization about the steady state; the exact definition is

$$L(\lambda)\mathbf{v} \equiv \frac{d}{dh}\,\mathbf{F}(\lambda, h\mathbf{v})|_{h=0}\,,$$

and the (i,j)th component of

$$M(\lambda)\mathbf{v} \equiv \frac{d}{dh}\,\mathbf{f}(\lambda, h\mathbf{v})|_{h=0}$$

is $\partial f_i/\partial u_j(\lambda, \mathbf{0})$. The matrix $M(\lambda)$ here is a generalized form of the Jacobian matrix A we encountered in appendix A.2.

Thus, our previous theorems (A.8) and (A.9) about linearized stability indicate that the steady state of (A.49) is stable if all eigenvalues of $L(\lambda)$ have negative real parts (or more generally, if the spectrum $\Sigma(L)$ of L is contained in the left half of the complex plane, bounded away from the imaginary axis) and unstable if the real part of at least one eigenvalue of $L(\lambda)$ is positive.

Now the heuristic discussion of bifurcation above may be considered more rigorously: *bifurcation occurs if a previously stable stationary solution becomes unstable at some critical value of the parameter* λ, which we can take without loss of generality to be $\lambda_c = 0$. We consider for now the simplest case: Let $\sigma(\lambda)$ be the principal eigenvalue of $L(\lambda)$, that is, the eigenvalue with greatest real part, and let it be a simple eigenvalue (that is, of multiplicity one in the characteristic equation for the operator); hence $\sigma(\lambda)$ is real, since complex eigenvalues of a real operator occur in conjugate pairs. Then bifurcation occurs if, at $\lambda = \lambda_c = 0$, this eigenvalue $\sigma(\lambda)$ increases past zero, so that the stationary solution $\mathbf{u} = \mathbf{0}$ is stable for $\lambda < 0$, and unstable for $\lambda > 0$. The above amounts to assuming the *transversality condition*, $\sigma(0) = 0$, $\sigma'(0) > 0$ [40].

Construction of Bifurcating Solutions It is then possible to construct the nontrivial solutions of (A.49) past bifurcation; so we look for the bifurcating steady solutions $(\lambda, \mathbf{u})$ of $\mathbf{u}_t = \mathbf{F}(\lambda, \mathbf{u}) = \mathbf{0}$.

Now for these novel stationary solutions, $\mathbf{u}$ may not be well-defined as a function of λ, so it is convenient to expand both as functions of a small parameter ε, by

$$\mathbf{u} = \sum_{n=1}^{\infty} \varepsilon^n \mathbf{u}_n \ , \quad \lambda = \sum_{n=1}^{\infty} \varepsilon^n \lambda_n \ . \tag{A.51}$$

where clearly $\mathrm{u}(\varepsilon = 0) = \mathbf{0}$, $\lambda(\varepsilon = 0) = \lambda_c = 0$.

Using these power series expansions, the bifurcating solution may be constructed explicitly, in terms of the eigenfunction $\phi(\lambda)$ of the linear operator $L(\lambda)$, corresponding to the eigenvalue $\sigma(\lambda)$ (and also in terms of the eigenfunction $\phi^*(\lambda)$ corresponding to the adjoint operator L^* of L in the appropriate Hilbert space H) [40]. Explicit expressions for $\mathbf{u}_1$, λ_1, then $\mathbf{u}_2$, λ_2 and so on may be derived, with the aid of some analysis of the behaviour of solutions in H, together with the theorem of the Fredholm alternative. As this derivation involves more detail than we are interested in here, the reader is referred to any standard text on bifurcation theory such as those mentioned above, for example Britton [40], or to Nicolis and Prigogine [276] for a less rigorous derivation. We merely state here the most significant result:

The most relevant fact to emerge from the bifurcation analysis is that the leading order term $\mathbf{u}_1$ in the constructed bifurcating solution $\mathbf{u}$ as a function of ε is *proportional to the eigenfunction* ϕ of $L(\lambda)$ evaluated at $\lambda = 0$, that is

$$\mathbf{u}_1 \propto \phi(0). \tag{A.52}$$

Thus to leading order (that is, in the vicinity of the bifurcation, for small ε), the qualitative behaviour of the solution may be obtained from the eigenfunction of the linear operator L evaluated at the bifurcation point; where, in a reaction-diffusion situation (where $L(\lambda) = M(\lambda) + D\nabla^2$), this eigenfunction typically depends on the geometry of the physical system.

The leading term λ_1 in the expansion (A.51) for the parameter $\lambda(\varepsilon)$ *on the bifurcating solution* is obtainable explicitly, but by a significantly more complicated expression (see [40, section 6.5]). The higher-order terms $\mathbf{u}_2$, λ_2 and so on may be found by iterations of the bifurcation analysis procedure, but beyond the next term or two the value of the results is generally not worth the effort involved: these terms give small corrections in the vicinity of the bifurcation point, and thus do not yield much useful qualitative information on the behaviour of the solutions immediately after bifurcation.

Behaviour Well Beyond Bifurcation — Mode Coupling For larger ε, the higher-order terms clearly become more significant and non-negligible; this corresponds physically to the fact that there is (nonlinear) interaction between the stable modes (eigenfunctions) (which correspond to negative eigenvalues σ) and the single linearly unstable, excited mode. In the language of synergetics (see section 3.1.4, and Haken [141]) the coefficients of the excited eigenfunctions are known as *order parameters*, and the coefficients of the damped (decaying, hence stable) modes are called *slaved variables*; the fundamental assumption of synergetics is that large numbers of degrees of freedom can be described by a limited number of collective modes with order parameters as coefficients, and that essentially all other modes may be neglected. Then higher-order corrections to the bifurcating solution may be obtained by taking the coupling between the growing part of the solution to those other eigenfunctions which are least damped (that is, have least negative eigenvalues) and which are strongly coupled to the excited part of the solution into consideration (see [92]). But such corrections are analytically time-consuming and intricate, and yield little new information in the immediate vicinity of bifurcation, so we do not proceed to consider them, rather referring the reader to discussions of bifurcation theory (for example, [40, 276]) or synergetics [141, 143].

Stability of Solutions — Supercritical and Subcritical Bifurcation A further issue which should not be neglected is the concern with the stability of the stationary bifurcating solutions $(\lambda(\varepsilon), \mathbf{u}(\varepsilon))$ which have been considered above. As usual, the stability analysis involves a linearization of the reaction-diffusion equations (A.49) about the solution of interest, which is in this case the bifurcating solution, no longer the homogeneous steady state. The quantity of interest in this case is the principal eigenvalue $\tilde{\sigma}(\varepsilon)$ (the extension of the $\sigma(\lambda)$ treated above, with $\tilde{\sigma}(0) = \sigma(0) = 0$, so clearly $\tilde{\sigma}$ is a simple, real eigenvalue) of the operator $\tilde{L}(\varepsilon)$ obtained by linearization about the bifurcating solution. The stationary solution is stable, as expected, if $\tilde{\sigma}(\varepsilon) < 0$ and unstable if $\tilde{\sigma}(\varepsilon) > 0$ beyond bifurcation.

The stability of the bifurcating solution may be assessed immediately with the aid of a 'Factorization Theorem' (see for example [40, theorem 6.81]): near bifurcation, that is as $\varepsilon \to 0$, this theorem says that

$$\tilde{\sigma}(\varepsilon) \sim -n\,\lambda(\varepsilon)\sigma'(0), \tag{A.53}$$

where n is the order of the first non-zero derivative of λ with respect to ε.

Thus, the bifurcating solution is stable if $\lambda(\varepsilon)$ on the bifurcating solution is positive, that is if the solutions bifurcate *supercritically*; whereas those solutions that bifurcate *subcritically*, with $\lambda < 0$ on the bifurcating solution $(\lambda(\varepsilon), \mathbf{u}(\varepsilon))$, are unstable. To see how this is possible, where λ was originally considered the bifurcation parameter *increasing* through zero, recall that we have now *constructed* $\lambda(\varepsilon)$ on the bifurcating solution, to give $\mathbf{u}(\varepsilon)$ implicitly depending on λ as a result.

An illustrative example, referring back to our first simple case of a pitchfork bifurcation, may clarify the distinction between supercritical and subcritical bifurcations:

For the case $\dot{x} = \lambda x - x^3$, the zero solution becomes unstable as λ increases through zero (the linearized equation is $\dot{x} = \lambda x$) and two new stable solutions, $x = \pm\sqrt{\lambda}$, for positive λ, form — this is a supercritical bifurcation. A minor modification of the above system gives, however,

$$\dot{x} = \lambda x + x^3; \tag{A.54}$$

here again the zero solution becomes unstable as λ increases through zero, so that $\lambda = 0$ is again a bifurcation point. In this case, however, the bifurcating solutions are $x = \pm\sqrt{-\lambda}$ for $\lambda < 0$, and are *unstable*. Here we have an unstable pitchfork bifurcation, a typical case of a subcritical bifurcation, where $\lambda < 0$ on the bifurcating solution is correlated with instability of the solution, as required in general by the factorization theorem (see figure A.1(b) on page 234).

To summarize the above discussion, we repeat the most significant point: *to first order, in the vicinity of the bifurcation, the form of the bifurcating solution is the same as that of the eigenfunction of the linearized operator at that point, corresponding to the eigenfunction which is drive unstable at bifurcation.* This vital aspect will be elaborated on later as we consider the initiation and nature of the patterns that may be formed (see [251]).

A.3.2 Further Bifurcation Phenomena

Temporal Symmetry-Breaking So far we have restricted our discussion to the study of steady (stationary) equilibrium solutions of the system $\mathbf{u}_t = \mathbf{F}(\lambda, \mathbf{u})$; at bifurcation, such solutions emerge when a (simple) real eigenvalue of the linearized operator $L(\lambda)$ crosses the imaginary axis. Qualitatively different behaviour is observed if a pair of complex conjugate eigenvalues crosses the imaginary axis at $\lambda = \lambda_c$ (necessarily simultaneously, as $L(\lambda)$ is real); in this case the bifurcating solutions may be periodic in time. The outcome is another form of symmetry-breaking, here in the *temporal* variable, as the symmetry of the time-independent 'forcing term' $\mathbf{F}(\lambda, \mathbf{u})$ is lost in the genesis of a time-periodic from a stationary solution. This bifurcation phenomenon is known as the *Hopf bifurcation*, or more generally by the historically more accurate appellation of Poincaré–Andronov–Hopf bifurcation; an important reference on the subject is the book by Marsden and McCracken [222].

The Hopf Bifurcation

A Simple Example As before, we do not proceed with a complete derivation for reaction-diffusion systems, but merely introduce the Hopf bifurcation phenomenon with the aid of a simple paradigmatic example, as we did earlier through the pitchfork bifurcation equation $\dot{x} = \lambda x \pm x^3$. As we now require two eigenvalues to cross the imaginary axis simultaneously, the problem is essentially two-dimensional in its simplest form. Thus, for an elementary example consider the system

$$\frac{\partial}{\partial t}\begin{pmatrix} x \\ y \end{pmatrix} = \begin{pmatrix} \lambda & -\omega \\ \omega & \lambda \end{pmatrix}\begin{pmatrix} x \\ y \end{pmatrix} - (x^2 + y^2)\begin{pmatrix} x \\ y \end{pmatrix} \tag{A.55}$$

where we may keep ω constant (for example choose $\omega = 1$) and treat λ as the bifurcation parameter. This system is more conveniently analysed in polar coordinates, so it is helpful to set $x = r\cos\theta$, $y = r\sin\theta$, or alternatively $r = \sqrt{x^2 + y^2}$, $\theta = \arctan(y/x)$. Then in these new variables the above system becomes

$$\begin{aligned} \dot{r} &= \lambda r - r^3, \\ \dot{\theta} &= \omega. \end{aligned} \tag{A.56}$$

The second equation has the solution $\theta = \omega t + \theta_0$ (where θ_0 is some initial phase angle) corresponding to steady rotation; it is the behaviour of the first equation that interests us. The

analogy with the stable pitchfork bifurcation equation considered earlier is immediately clear, but we note that we are here dealing with the amplitude of oscillations, so that r must be non-negative. Thus for $\lambda < 0$, the only equilibrium solution is $r = 0$, that is the steady state solution at the origin $x = 0$, $y = 0$, which is stable (linearized equation $\dot{r} = \lambda r$). On the other hand, for $\lambda > 0$, the trivial stationary solution becomes unstable, and the solution $r = \sqrt{\lambda}$ attains stability. This corresponds, in the original Cartesian variables, to the bifurcated solution $x = \sqrt{\lambda}\cos(\omega t + \theta_0)$, $y = \sqrt{\lambda}\sin(\omega t + \theta_0)$, that is an isolated, stable, *periodic solution* about the unstable critical point at the origin. All trajectories in the phase plane converge to this stable isolated periodic solution, or limit cycle, which has bifurcated from the trivial solution; thus we have a breaking of temporal symmetry, as the system is now endowed with a characteristic time scale corresponding to the period of oscillations — this is the simplest case of temporal self-organization.

The Existence of Periodic Solutions As before, we do not here present a full generalized treatment of the Hopf bifurcation or of periodic solution behaviour (refer to one of the books mentioned above, for example [40, 222, 251], for a more detailed discussion); it suffices to state the main result. The existence of periodic solutions may be deduced with the aid of the *Hopf bifurcation theorem*, which we state for the two-dimensional case — the extension to higher dimensions is straightforward:

Theorem A.10 (Hopf Bifurcation Theorem) *Consider the two-dimensional bifurcation problem* $\dot{\mathbf{u}} = \mathbf{f}(\lambda, \mathbf{u})$ *where* $\mathbf{f}$ *is analytic (this condition may be weakened) and* $\mathbf{f}(\lambda, \mathbf{0}) = \mathbf{0}$*; and assume that the complex conjugate eigenvalues* $\sigma(\lambda)$ *and* $\bar{\sigma}(\lambda)$ *of the linearization of* $\mathbf{f}$ *cross the imaginary axis from left to right at* $\lambda = 0$*, so that* $\sigma(0) = i\omega_0 \neq 0$*,* $\mathrm{Re}\,\sigma'(0) > 0$*. Then small amplitude periodic solutions bifurcate from the trivial solution as we pass through* $\lambda = 0$*; that is, there exists a periodic solution of* $\dot{\mathbf{u}} = \mathbf{f}(\lambda, \mathbf{u})$ *for* λ *in some neighbourhood of* $\lambda = 0$ *and* $\mathbf{u}$ *in some neighbourhood of* $\mathbf{0}$*, with approximate period* $T = 2\pi/\omega_0$ *for small* λ*.*

For the rather complicated proof of this important theorem, see any of the texts cited above.

Note that the theorem gives no direct indication of the stability of the periodic solutions, but only confirms their existence. To deduce stability, for two-variable systems it is generally simplest to obtain a qualitative picture using phase-plane analysis; for general systems, further analysis yields a complicated formula involving a combination of several second and third derivatives of $\mathbf{f}$, which enables stability to be determined. As for one-dimensional bifurcations above, the basic result emerging from the analysis is that (if $\mathrm{Re}\,\sigma'(0) > 0$) supercritical bifurcations are stable, with $\lambda(\varepsilon) > 0$ on the bifurcating solution, and those that bifurcate subcritically, with $\lambda(\varepsilon) < 0$, are unstable.

Extensions of Bifurcation Theory to Higher Dimensions

How may these elementary results on the bifurcation of periodic solutions obtained in two dimensions be extended to the physically more relevant cases of several, or even infinitely many (in the case of systems depending on a continuous space variable, such as reaction-diffusion systems) dimensions? Intuitively we might anticipate that the behaviour would be considerably

more complicated, but this is not necessarily so — in fact, typically higher-dimensional systems may essentially be reduced to the cases studied above.

To see this, note that as usual we need to consider the linearization of our dynamical equations, such as (A.49), about the steady state in the vicinity of the bifurcation point $\lambda = \lambda_c$, and consider the eigenvalues $\sigma(\lambda)$ of the linearized operator $L(\lambda)$. Then as discussed previously, 'typically' (generically, in more formal terms) one of two situations will occur at bifurcation — either a simple (real) eigenvalue, or a pair of complex conjugate eigenvalues, will cross the imaginary axis to Re $\sigma > 0$ at $\lambda = \lambda_c$, while the other eigenvalues remain strictly in the left half of the complex plane (that is, they retain negative real parts). Hence the behaviour of the system with respect to the eigenvalues that cross the imaginary axis is still stable, and the characteristic bifurcation behaviour is as before: in the first case (simple real eigenvalue becomes unstable), the bifurcating solutions will be non-oscillatory — we have considered this scenario in more depth already — whereas in the second case, beyond bifurcation oscillatory (time-periodic) solutions in the form of limit cycles are observed.

The heuristic discussion above may be elaborated in slightly more general terms, using arguments which we have already referred to: The solution of a system such as (A.49) may be written in terms of the eigenfunctions of the linearized operator, for example as in (A.14). Then the components for which the eigenvalues σ are negative, decay with time — these correspond to the stable directions, which are 'uninteresting'. Thus the behaviour of the system may essentially be described by the one or two components which become unstable, and initially grow exponentially (before being bounded by nonlinearities). In general function spaces, we may define stable, centre and unstable manifolds for the dynamical system solution behaviour, with the interesting behaviour occurring on the centre manifold containing components driven unstable at bifurcation [138].

Universality in Bifurcation Behaviour: Normal Forms For a general n-dimensional system, a set of quantities may be defined (which correspond to the reduction of the system vector $\mathbf{u}$ to the centre manifold) which, once an appropriate normalization, or change of variables, is performed, satisfy a closed set of equations having a universal structure, called a *normal form*. Effectively, then, the original multivariable dynamics is decoupled into a single equation or pair of equations (the latter for bifurcation to a periodic solution) which satisfy the normal form equations and give information on the bifurcation, with the remaining $n-1$ or $n-2$ equations being irrelevant as far as bifurcation is concerned, as they correspond to decaying behaviour on the stable manifold. The variables satisfying the bifurcation equations, that is the coefficients of the excited eigenfunctions in the normalization, are denoted as the order parameters, whereas the coefficients of the damped modes are the slaved variables, as defined above.

The possible normal form equations, for the case when a real eigenvalue crosses Re $\sigma = 0$ to give a stationary solution, are [277]

$$\frac{du}{dt} = (\lambda - \lambda_c) - \mu u^2 \tag{A.57}$$

or

$$\frac{du}{dt} = (\lambda - \lambda_c)u - \mu u^2 \tag{A.58}$$

or

$$\frac{du}{dt} = (\lambda - \lambda_c)u - \mu u^3 \tag{A.59}$$

(the last of these is the pitchfork bifurcation treated above, which in fact turns out to be structurally unstable); and for the second case, with two complex conjugate eigenvalues crossing the imaginary axis, the normal form equation is as for the Hopf bifurcation:

$$\frac{d}{dt}\begin{pmatrix} u \\ v \end{pmatrix} = \begin{pmatrix} \lambda - \lambda_c & -\omega \\ \omega & \lambda - \lambda_c \end{pmatrix}\begin{pmatrix} u \\ v \end{pmatrix} + \mu(u^2 + v^2)\begin{pmatrix} u \\ v \end{pmatrix} \tag{A.60}$$

where μ is some coefficient, and ω is the frequency of oscillations at the bifurcation point.

The idea of reduction of the system to a few order parameters is frequently associated with Landau in connection with the theory of phase transitions; in the mathematical literature it is often referred to as the Lyapunov-Schmidt procedure, or as centre manifold theory. It is also the basis of Haken's method of 'synergetics' [141], that large numbers of degrees of freedom may be described by a limited number of collective modes with order parameters as coefficients, and that essentially all other modes may be neglected — we have already referred to this approach (see section 3.1.4).

The essential lesson is that when considering higher dimensions or infinite dimensions, such as reaction-diffusion systems, it is typically not necessary to consider radically new concepts, as the bifurcations discussed above (together with a limited number of other less common characteristic cases) will still apply. It may be said (following Nicolis [274]) that the stable normal forms for bifurcation and hence the beginnings of the generation of complex structure, constitute a limited number of 'archetypes' of complexity. (Catastrophe theory [344] provides an alternative, though to some extent analogous, approach to the classification of the generic possibilities for structure formation — see section 6.1.)

Other Possible Solution Behaviours

We have so far considered only the behaviour in the immediate vicinity of the bifurcation point, and we have seen that stationary (possibly space-dependent) and time-periodic solutions may result. What happens beyond this point? Before any general answer can be given, the most pertinent comment is that the analysis of the behaviour of the bifurcated equilibrium solution is considerably more difficult, as in general we do not have an exact analytic expression for the solutions, and so we have to resort to approximate or numerical methods.

In large classes of systems, the diversification of behaviour through bifurcation does not cease at the first transition (the primary bifurcation); instead, one can observe complicated bifurcation cascades leading to secondary bifurcations (that is, bifurcations off a bifurcated

solution), tertiary bifurcations, and so on, in some cases eventually leading to the bifurcation of chaotic attractors. Such secondary and higher-order bifurcations appear as the parameter λ increases and other eigenvalues beyond the initially unstable ones also cross the imaginary axis; nonlinear coupling between the various unstable eigenfunctions may now result. Exceptional situations (nongeneric except for systems with certain symmetries) may also occur, where three or more branches of eigenvalues cross the imaginary axis simultaneously at $\lambda = \lambda_c$ — this leads to the interaction of bifurcating solutions, again giving higher-order bifurcation phenomena. For each such case, the behaviour of the bifurcating branches again takes place in a phase space of reduced dimensionality, the centre manifold, and it is possible to reduce the system to a normal form; but now the construction of the normal forms becomes much more complicated, and its universality can no longer be guaranteed [274].

As alluded to above, new types of attractors, in addition to the standard forms of fixed points, limit cycles or tori (multiperiodic oscillations) may be created, including some global bifurcation phenomena such as chaotic attractors (if the normal form equations contain at least three coupled order parameters, as in the classical Lorenz system of three coupled ordinary differential equations). Chaotic attractors are characterized by stretching along unstable directions and folding along the stable manifolds, together with a fractal geometry; they correspond to complete loss of predictability and sensitivity to initial conditions [318]. Nevertheless, they may arise by a similar process of bifurcation that produces pattern and order, as discussed above. The range of phenomena attainable through successive bifurcations is well demonstrated in the variety of possible solution behaviours in the Belousov-Zhabotinskii equation (see appendix B).

For the case of spatially distributed systems such as reaction-diffusion equations, possible bifurcations may include *spatial symmetry-breaking* phenomena, as already asserted; in this case, the order parameters represent the amplitudes of the dominant modes appearing in an expansion of the state variables in a Fourier Series (or more generally, in a complete orthogonal series of independent functions compatible with the symmetry properties of the system and the boundary conditions; that is, the eigenfunctions of the Laplacian operator in Ω). Such bifurcations then lead to spatial self-organization, which constitutes a major focus of our interest; a discussion of the types of patterns that may be generated, that is the solution behaviour beyond bifurcation, for a two-variable reaction-diffusion system is the subject of section A.4.

A.3.3 Other Analytical Techniques

We have considered some aspects of bifurcation theory and techniques which may be used to understand the 'typical' behaviour of reaction-diffusion equations and others exhibiting spatial or temporal symmetry-breaking, that is aspects of self-organization, beyond a bifurcation point $\lambda = \lambda_c$. There is a large variety of other analytical techniques that may be used to study such systems (for a brief discussion of some aspects of numerical methods, see appendix A.5). We refer here to work on reaction-diffusion systems, but analogous techniques apply to other comparable nonlinear dynamical systems. The range of methods available can only be touched on here.

As reaction-diffusion systems are nonlinear, exact solutions may not be obtained, and so special features of the equations have to be exploited and *approximate techniques* used to obtain estimates and indications of the qualitative behaviour of such systems. Such approximate tech-

niques often reveal novel aspects of the behaviour of reaction-diffusion systems that cannot occur in linear systems. Many approximate methods can usually be refined to any desired degree of accuracy by extending the analysis to higher-order terms (although often with considerable effort, exceeding the potential benefit). Furthermore, there can be in principle no real harm in the use of approximate methods, since the model equations being studied are invariably approximations to the real physical situation anyway, so that anything better than an approximation is in general impossible to obtain. Such considerations motivate our study of approximate methods.

The standard approximate techniques involve some form of *asymptotic expansions*, generally involving the expansion of the solution as a power series in some small parameter ε (or in Λ^{-1}, where Λ is a large parameter). Such methods are used in situations where one or more of the (nondimensionalized!) parameters of the system is small, as the smallness of the parameter may be utilized to indicate the *dependence* of the solutions on that parameter (whereas numerical solutions are necessarily restricted to *particular* sets of the parameter values). Frequently, the context is that of small changes, or perturbations, to a problem to which the solution is known or well-understood, by the addition of extra terms or a slight change in one of the parameters of the problem. Various asymptotic or perturbation methods are available, both for *regular perturbation problems* (where the perturbed solution is in some well-defined sense 'close' to the solution of the unperturbed problem) and for *singular perturbations* (where the solution is not close).

Regular Perturbation Methods Asymptotic methods for regular perturbation problems are used *inter alia* for finding approximations to the amplitude and period of limit cycle solutions, using techniques such as the method of renormalization, the two-timing method (treating a 'fast' and a 'slow' time variable as independent), and averaging methods. Such methods have been used for example to demonstrate the existence of plane wave and spiral wave solutions to reaction-diffusion equation systems (see [40]).

Singular Perturbation Problems Singular perturbation problems present different challenges, and correspondingly appropriate methods are used, such as the method of matched asymptotic expansions. Such techniques have proved indispensable in the study, for example, of *threshold phenomena* (where one may have a stable steady state, but the kinetics are such that some perturbations from the steady state undergo a large excursion through phase space before the trajectory settles down again to the steady state). If diffusion is included in the system, singular perturbation analysis has been used to demonstrate the possibility of wave fronts and solitary travelling wave solutions in such systems, and to obtain estimates, to leading order in the asymptotic expansion, of the speed and thickness of the leading and travelling wavefronts. The analysis (corroborated by numerical simulations) indicates that these travelling waves are stable solutions of the equations, and that they are *trigger waves*, in that they arise from a stable steady state; thus they are not initiated by arbitrarily small (statistical) fluctuations, but can only be triggered by a finite perturbation from the steady state — the reaction medium (in the case of a chemical system) in this case is said to be *excitable*; the Belousov-Zhabotinskii reagent furnishes an example of such a system.

We have here merely *mentioned* some of the available approximate methods available for the study of reaction-diffusion and allied systems; for an introduction to the application of such

techniques to the study of reaction-diffusion systems, see in particular the books by Britton [40] and Murray [245, 251], and references therein.

A.4 Aspects of Pattern Formation and Pattern Selection in Reaction-Diffusion Systems

We have so far considered reaction-diffusion systems in some detail, focussing on aspects of the solution behaviour specifically of systems of parabolic partial differential equations, as well as on more general considerations of stability, bifurcation and spatial and temporal symmetry-breaking appropriate to self-organizing systems in general. The intention has been to outline and briefly demonstrate some techniques of analysis that are pertinent to general dynamical models describing the origin of order and pattern, and to apply them to the most popular and best-studied, indeed paradigmatic self-organizing system. In this way support for the conceptual possibility of self-organization in realistic chemical and physical systems has been presented, in the demonstration that symmetry-breaking bifurcations can occur. Thus the idea of the generation of pattern, the origin of order and organization, and ultimately the genesis of complexity has been established, at least in principle.

If, however, we wish to develop models directly applicable to an experimental situation in, say, developmental biology, then the questions we need to ask of the equations are not conceptual ones of the general, abstract 'potentiality for self-organization', but rather more concrete questions in order to aid in the establishment of a detailed correspondence between theory and experiment, and hence to assist in the design of experiments. Thus we seek to know, for example, what kinds of patterns can be formed (and whether these correspond to those observed); how long they take to form (and whether these time scales are feasible); what the effects of the scale and geometry of the domain under consideration are; for what parameter ranges pattern formation occurs (are these ranges realistic, and robust enough to maintain pattern-forming potential in the face of inevitable environmental perturbations?); and analogous pertinent characteristics of the models, in order to expedite the comparison with experiment.

Discussions of the 'realism' of proposed models tend to accompany most applications of reaction-diffusion systems in the literature. Furthermore, a lengthy and detailed exposition of some issues to be considered in the comparison with experiment is given in Murray's book [251], to which the reader is referred (and on which much of this discussion is based). Thus we will here present only an outline of some of the methods used for the analysis and comparison of models, and a mention of some general characteristics of reaction-diffusion-generated patterns beyond the spatial symmetry-breaking bifurcation.

A.4.1 Nondimensionalization, Parameter Dependence and the Dispersion Relation

The Value of Nondimensionalization

A vital step to facilitate the analysis of the dynamical equations describing a reaction-diffusion (or other) system, once the chemical kinetic, mechanical and other influences have been quantified and incorporated into the model equation formalism, is nondimensionalization. This pro-

ceeds by the introduction of characteristic length and time scales, and the consideration of combinations of the parameters that give dimensionless groupings, and yields the reduction of the dimensional dynamical variables (describing for example chemical concentrations), space and time variables, kinetic, diffusion and other parameters to nondimensional (unitless) form (for an example of the application of this technique, see section 3.2.3).

The need for nondimensionalization has already been referred to in several places, together with some indication of its advantages. Essentially, once nondimensionalization has been performed, the sizes of variables and parameters (and hence the results of calculations) are not dependent on the units used. Consequently their *relative* sizes may be assessed, which is crucial for the use of asymptotic techniques, and for the general assessment of which terms are 'small' and may be neglected for computational simplicity. A second virtue is that, by means of nondimensionalization, different reaction-diffusion systems may be written in the same general form, which facilitates the comparison of models; for example, for the linear stability analysis performed above, in section A.2, it was found convenient to reduce the general two-variable reaction-diffusion system to the nondimensional form (A.19)–(A.20)

$$\left.\begin{aligned} u_t &= \gamma f(u,v) + \nabla^2 u \\ v_t &= \gamma g(u,v) + d\nabla^2 v \end{aligned}\right\} \tag{A.61}$$

(clearly, this corresponds to the form in (A.49) if we take

$$\mathbf{u} = \begin{pmatrix} u \\ v \end{pmatrix}, \quad \mathbf{F(u)} = \gamma \begin{pmatrix} f(u,v) \\ g(u,v) \end{pmatrix}, \quad D = \begin{pmatrix} 1 & 0 \\ 0 & d \end{pmatrix}).$$

As a result, the general linear stability analysis performed above is applicable to an entire range of different mechanisms and reaction-diffusion systems. We shall largely make use of this form (A.61) for the remainder of this section.

The effect of nondimensionalization is to emphasize those aspects of the system which are independent of the specific units and scales used, that is those which are in some sense *intrinsic* to the system. For certain systems, different nondimensionalizations are often possible, which may bring out different features of the system. The analysis of general reaction-diffusion systems and their pattern-formation properties reveals that the behaviour of the system depends critically on certain parameters; for example, in our linear stability analysis above, we used d as the bifurcation parameter. But these crucial parameters are generally dimensionless groupings or combinations of the dimensional parameters — for example d is the ratio of the diffusion coefficients, $d = D_v/D_u$ — so that the effect of the variation in one parameter may be counteracted, or alternatively simulated, by variation in another, hence allowing a full appreciation of the pattern-forming properties of each parameter. For example, the increase in d which leads to bifurcation and spatial symmetry-breaking may be achieved by an increase in D_v or a decrease in D_u, or some combination thereof. Thus we are led to the concept of *equivalent effects via parameter variation*, that is that the variation of different parameters may have comparable (interchangeable) effects with respect to pattern-forming properties [251, 287].

Parameter Dependence and the Turing Space

In the process of performing a linear stability analysis for the general nondimensional system (A.61) (see appendix A.2), we obtained a number of conditions on the kinetics of the system and

the ratio of diffusion coefficients, in order for diffusive-driven, or Turing, instability to be possible — that is, for the system to be stable to spatially homogeneous perturbations, but unstable to spatially inhomogeneous perturbations of the appropriate wavelength. These conditions were scattered above as inequalities (A.29), (A.30), (A.39) and (A.42); it is convenient to gather them together here:

$$\begin{aligned} trA = f_u + g_v &< 0, \\ |A| = f_u g_v - f_v g_u &> 0, \\ d f_u + g_v &> 0, \\ \frac{(d f_u + g_v)^2}{4d} &> |A|; \end{aligned}$$

where all partial derivatives are evaluated at the homogeneous steady state (u_0, v_0), and we again (as in appendix A.2) denote the Jacobian matrix by A.

The specific forms of $f(u,v)$ and $g(u,v)$ depend on the kinetics of the particular reaction-diffusion system under consideration, which defines the detailed forms of the above inequalities. Furthermore, the kinetic parameters for the mechanisms considered appear in f and g, and also specify the steady state coordinates u_0 and v_0, so that ultimately the above inequalities give a set of relations between the system parameters for pattern formation to be possible. In parameter space, they define a set of curves, given in general parametrically, such that the enclosed domain permits pattern formation; these curves define the parameter space, or '*Turing space*' [249], wherein the steady state can be diffusionally driven unstable and hence permit the creation of spatial patterns. Note that the parameters appearing in the specification of the Turing space are dimensionless, as discussed above. In general this means that there are several ways the original dimensional parameters may be varied to enter the pattern-forming regime; so that it is the orchestration of several effects in general which produces pattern, not just a single effect [288]. It will become apparent later that the requirement that the parameters lie within the Turing space is a *necessary*, but not *sufficient* condition for patterning; scale and geometry also play a crucial role.

Implications of the Turing Space The concept of the Turing space may be used as a means of comparing mechanisms for their plausibility with respect to potential applications. If the Turing space is small, then parameters have to be *fine-tuned* to fall within the appropriate domain and hence allow pattern formation; this appears relatively unlikely, in the face of the inevitable random perturbations prevalent in the real world. Thus a large Turing space, which correlates with a more robust kinetic mechanism, allowing pattern formation over a greater range of parameters, seems to be a positive attribute of models, so that Turing space considerations may be used to aid in the theoretical discrimination between mechanisms. Murray [249] has evaluated the Turing spaces for a number of reaction-diffusion mechanisms and hence compared their domains of Turing instability.

While on the one hand the stability of a large Turing space seems important to potential applications in, say, biological development, on the other hand the sensitivity of patterning processes to parameter magnitudes may have a considerable evolutionary significance: an otherwise apparently minor mutation could modify a parameter in such a way that the reaction-diffusion system is pushed into the Turing space. Hence the system is 'thrown into pattern-generating

mode', and profound alterations in morphology could ensue virtually at one stroke, as the 'switching on' of the patterning mechanism allows the creation of novel structure [268]. Depending on whether we are interested in reproducible patterning in development, or creation of new structures in evolution, we may be led to 'favour' a small or large Turing space. Of course, distinctions made between models on the basis of Turing space (or analogous) considerations are at best theoretical, to aid in finding models and reaction systems which simulate the observed behaviour for the duration of our ignorance of the actual biochemical processes involved. The actual mechanisms utilized in biology are 'chosen' by natural selection as those which achieve the required results, not on the basis of externally imposed theoretical considerations.

The Dispersion Relation

As a first attempt at gaining an appreciation of the solution beyond bifurcation, we may consider the solution of the linearized reaction-diffusion equation, which may be written in the form (A.14). In this formulation, the solution depends on the functions ϕ, the eigenfunctions of the Laplacian on Ω; strictly speaking, as it derives from a linearization, this form is only valid immediately beyond primary bifurcation, in the vicinity of the homogeneous steady state. The expansion (A.14) may be written as a sum over the allowed wave numbers k, as

$$\mathbf{w}(\mathbf{x},t) = \sum_k c_k e^{\sigma t}\phi_k(\mathbf{x}). \tag{A.62}$$

(a justification for this formulation will be presented below).

The Role of the Eigenvalue σ In the expression (A.62), the modes ϕ are weighted by the time-dependent term $\exp(\sigma t)$, so that it is immediately apparent that the positivity or negativity of σ plays a crucial role in the pattern beyond bifurcation; in particular, if all values of σ are negative, then $\mathbf{w}$ will be damped to zero, perturbations from the uniform solution die out, and the homogeneous steady state is stable.

This determinative role for σ is in accordance with all our aforegoing discussions: In the general bifurcation analysis of section A.3.1, the stability of the homogeneous steady state of (A.61) was shown to depend on the eigenvalues $\sigma(\lambda)$ of the linearized operator of the kinetic terms, which we wrote in (A.50) as $L(\lambda)$, where λ was the bifurcation parameter; here the initial bifurcation, or loss of stability of the steady state, occurred as one of the eigenvalues σ became positive, leading to stable stationary solutions. In the alternative, but equivalent formulation of section A.1.3, we considered the eigenvalues σ of the matrix $A_n = \gamma A - \mu_n D$, where the (non-negative) μ_n are the eigenvalues of $-\nabla^2$ on the domain Ω and appropriate boundary conditions. Whichever derivation of the stability relationships one uses (ultimately, all our different approaches may be shown to be equivalent), the significant part played by the eigenvalues is readily apparent.

An expression for the eigenvalue may be obtained from equation (A.31), that is,

$$\det(-\gamma A + k^2 D + \sigma I) = 0. \tag{A.63}$$

Here k is introduced by writing the non-negative eigenvalues of the Laplacian operator as $\mu_n = k_n^2$, and then letting k stand for such a general k_n; k is a *wave number*, as is readily apparent from

the form of the corresponding eigenfunctions of the Laplacian in one dimension with zero flux boundary conditions, $\cos(kx)$. The equation (A.63) then yields a relation of the form $\sigma(k^2) = 0$. This relation enables us to consider the eigenvalues σ as functions of k^2; this is by no means intended to imply that we regard k as the bifurçation parameter — that role is taken by, for example, γ or d — but that k^2 also depends on the bifurcation parameter, and that at the point where $\sigma > 0$ initially, at bifurcation, it corresponds to a certain value of k^2, thus defining the eigenfunction which is initially driven unstable.

For a finite domain, the values for k are discrete, as the Laplacian operator has a discrete spectrum; hence each allowed value of k corresponds uniquely to an integer n. It follows that the eigenfunctions ϕ may be indexed by k, which is equivalent to being identified by n, and that hence we may write the expansion (A.62), which in (A.14) was a sum over integers n, as a sum over values of k; this motivates our form of (A.62).

Ranges of Unstable Wave Numbers It is apparent from the expansion (A.62) that the dominant contributions to the solution **w** beyond bifurcation are those growing, excited modes ϕ_k for which Re $\sigma(\mathrm{k}^2) > 0$, as all other modes decay to zero exponentially (the initial exponential growth of the excited modes is bounded and damped eventually by nonlinear interactions — it is important in the general analysis of a reaction-diffusion system to confirm the existence of an invariant set in the positive quadrant, to verify that unbounded growth of excited modes will indeed not occur). Thus the pattern-forming potential (as measured by its linearized approximation) of a reaction-diffusion system may usefully be studied by an analysis of the function Re $\sigma(\mathrm{k}^2)$, which yields the *dispersion relation*. The graph of this dispersion relation, obtained by plotting Re σ against k^2, permits an immediate visual interpretation of the likely patterns that may be formed — see figure A.2.

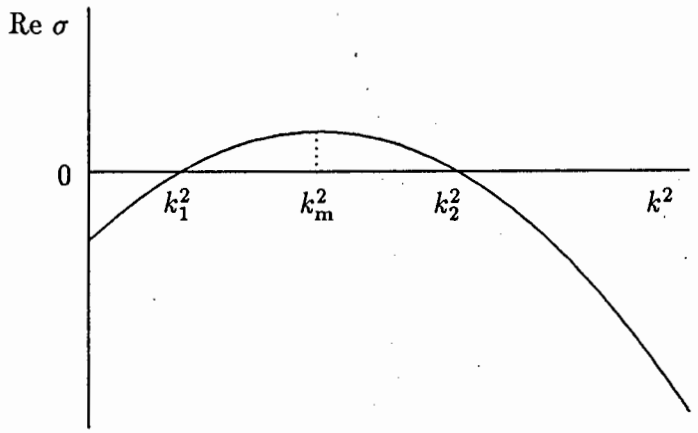

Figure A.2: Dispersion relation, or plot of the larger of the eigenvalues, Re σ, as a function of k^2, obtained from the solution of equation (A.33); the dispersion relation is shown for the situation beyond bifurcation, that is for $d > d_c$, so that the system is linearly unstable for the range of wave numbers $k_1^2 < k^2 < k_2^2$.

The dispersion relation shown in figure A.2 is the standard, or 'vanilla' [251], dispersion relation obtained from the general linear stability analysis of a two-variable reaction-diffusion system. (Dispersion relations may also be obtained for other hypothetical pattern-forming mod-

els — see appendix C.2 for the mechanical models [257]; they are interpreted similarly, although the diversity of dispersion relations is much greater, and some of the corresponding pattern-forming phenomena are much less well-understood.) The vanilla dispersion relation displays a number of characteristic and important features:

- There is a *finite band* of wave numbers k (and corresponding wavelengths) for which Re $\sigma(\mathrm{k}^2) > 0$. Note that the boundedness of the domain restricts the possible values of k to a discrete set compatible with the geometry. Then it is precisely those discrete values of k which fall within the 'unstable range', which indicate unstable, growing modes which contribute to the final pattern. The linear stability analysis enables the determination of the range of values of k for which instability occurs; instability is possible for

$$\begin{aligned} \gamma\Phi_1 = k_1^2 &= \frac{\gamma(df_u + g_v) - \{(df_u + g_v)^2 - 4d|A|\}^{1/2}}{2d} < k^2 \\ &< \frac{\gamma(df_u + g_v) + \{(df_u + g_v)^2 - 4d|A|\}^{1/2}}{2d} = k_2^2 = \gamma\Phi_2. \end{aligned} \quad \text{(A.64)}$$

  (This equation defines k_1 and k_2, as well as the functions Φ_1 and Φ_2).

- The spatially featureless mode $k = 0$, corresponding to infinite wavelength, is stable (Re $\sigma < 0$); this is as we required for stability to homogeneous perturbations (section A.2.1).

- For arbitrarily large k, or very small wavelength, the system is again stable, so that there cannot be pattern formation on an arbitrarily small scale.

- The dispersion relation has a single peak; as the magnitude of Re σ gives the initial rate of growth or decay of the various modes, from equation (A.62), the fastest growing or 'most unstable' mode may be identified as that which is closest to the peak. The corresponding value of k is given by k_m in (A.41); it is

$$k_\mathrm{m} = \gamma\frac{df_u + g_v}{2d}.$$

  This is the wave number that first becomes unstable, at $d = d_c$, the bifurcation point, and the wave number which is likely to dominate the final pattern as it corresponds to the fastest growing mode.

If only a single value of k falls within the unstable range, then from (A.62) the pattern for large t may be expected to resemble the corresponding eigenfunction ϕ_k, as all other modes decay. Indeed, in the immediate vicinity of bifurcation the truth of this conjecture is attested by the result of our analyses in section A.3: that for bifurcation of a stationary solution from a homogeneous steady state, the bifurcated solution corresponds, to first order, to the eigenfunction of the linearized operator evaluated at the steady state, corresponding to the eigenvalue which is driven unstable. That is, if $\sigma(k^2) > 0$ for some k, then the solution will 'look like' ϕ_k.

Superposition of Modes If Re $\sigma(\mathrm{k}^2) > 0$ for a finite range of values of k, it may happen that several allowed modes may become unstable, for $k_1 < k < k_2$. In this case, as we have

seen before, there is a mode, closest to the peak of the dispersion relation, which is fastest-growing, so that we might expect that mode to dominate the final pattern. However, as soon as several modes become unstable, we are distant from the primary bifurcation point, and so our bifurcation analyses performed above provide relatively little rigorous indication of the final solution behaviour. Indeed, as we saw in section A.3.2, as a second mode becomes unstable, secondary bifurcation occurs, and we can expect nonlinear interaction between modes, possibly the effect of higher harmonics; and also the non-negligible influence of some 'slaved' modes (which decay linearly, but relatively slowly, and thus influence the final pattern).

A general nonlinear analysis for the evolution of finite-amplitude spatial patterns is lacking, owing to the complications that can result from the coupling between modes, and we have analytical results only in the vicinity of the bifurcation point, such as singular perturbation analyses, to fall back on. To study the situation far from bifurcation, in the range with several unstable modes, it is necessary at present to resort to numerical methods (some of these methods are discussed briefly in appendix A.5). The results indicate that, at least for small k, the fastest-growing mode is still a fairly good predictor of the final pattern, but that there is a strong dependence on initial conditions, such that a polarity or dominant mode in the initial conditions can determine the mode that is selected; also, the nonlinear couplings between modes become evident when several modes are excited. We will consider mode selection in more detail below.

A.4.2 The Role of Scale and Geometry

So far, the discussion has considered largely mode selection in the context of the growth of excited modes, or eigenfunctions of the Laplacian, and has concerned itself with the nonlinear coupling between modes and other abstract issues, without determining more exactly the *nature* of these eigenfunctions. So what are the types of patterns that may be formed, and what is the influence of geometry and scale?

Geometric Constraints in One Dimension

We consider firstly a reaction-diffusion system in one dimension, both as it provides considerable analytical simplification, and to illuminate the major features. The space variable in this case is $x \in [0, l]$ for some l. Then for zero flux boundary conditions, the eigenfunctions ϕ_n of the Laplacian $-\nabla^2 = -\partial^2/\partial x^2$ in this domain Ω are $\cos(n\pi x/l)$, with corresponding eigenvalues $(n\pi/l)^2 = k^2$, where n is a non-negative integer (for zero Dirichlet boundary conditions, the eigenfunctions would be $\sin(n\pi x/l)$). For such cosinusoidal eigenfunctions, the wavelength is $\lambda = 2l/n$, so that $k = n\pi/l = 2\pi/\lambda$; that is, k corresponds to the *wave number*, thereby justifying all our previous references to k using that terminology.

The Chemical Wavelength Immediately beyond the bifurcation point, at some critical $d = d_c$ (if we take d as the bifurcation parameter, as in section A.2), there is a corresponding critical k given by $k_c = \gamma(df_u + g_v)/2d$, as we have seen already; this gives the wave number of the mode that initially becomes unstable, with a pattern of the form $\phi_k(x) = \cos k_c x$ and a wavelength $\lambda_c = 2\pi/k_c$. This constitutes an *intrinsic chemical wavelength* for the pattern, which depends only on the chemical kinetics and the diffusion coefficients (the γ in the expression for k_c could

be normalized to 1 by choosing an appropriate length scale, so its presence in the expression for λ_c does not refute the existence of a wavelength independent of the domain). This is in contrast to the hydrodynamic situation, such as in the Bénard experiment (see section 3.1.2), where the wavelength depends on the geometry — it is characteristic of reaction-diffusion systems and generally of chemical self-organization in dissipative systems that there is this intrinsic *length scale*.

Constraints due to Boundary Conditions On the other hand, the expressions we obtained above for the eigenfunctions and eigenvalues of the Laplacian, subject to the geometry and boundary conditions, immediately indicate a restriction on the permitted values of k, which is why we have constantly mentioned the requirement of 'allowed' wave numbers. The geometric constraints dictate that not *any* wave numbers are possible, as a half-integral number of wavelengths must fit exactly into the domain. If this condition is not satisfied, then no pattern can form in spite of our kinetic requirements for spatial symmetry-breaking being satisfied.

Hence there are two requirements for pattern formation to be possible for a given k:

1. Re $\sigma(\mathrm{k}^2) > 0$ — the kinetic condition, that the homogeneous steady state must be unstable to inhomogeneous perturbations;
2. $k = n\pi/l$ for some integer n — the geometric condition, that the pattern must *fit into* the domain, implying that only a discrete range of wave numbers is permitted.

As we move further from bifurcation, so that Re $\sigma(\mathrm{k}^2) > 0$ for a range of k values, pattern will be formed for a wave number that satisfies the boundary condition, to give a wavelength that is as close as possible to the critical wavelength, but is adjusted to fit an integral number of waves into the domain. The geometrical conditions are constraints especially if the domain is small, so that it is difficult to fit the boundary conditions; if the domain size is large, there are essentially no real geometric restrictions (as the allowed wave numbers $n\pi/l$ are close to each other for large l), so a wavelength close to the intrinsic chemical wavelength arising from the kinetics may be chosen. We may say that geometrical considerations *constrain*, but do not *specify*, the length scales of pattern formation — in contrast to the case for hydrodynamics, where the scales actually depend on the domain size directly.

Our previous discussion concerning the situation when Re $\sigma(\mathrm{k}^2) > 0$ for a finite range of k values, $k_1 < k < k_2$, and several modes may be excited, carries over when these geometrical considerations are taken into account: The fastest growing mode must be expected to be that *allowed* mode for which $\sigma(k^2)$ is a maximum, that is for that value of n for which $k = n\pi/l$ is closest to the intrinsic value k_m. More generally, using the definitions of Φ_1 and Φ_2 above (A.64), the modes which are driven unstable are those for which some integer n satisfies the inequalities

$$\gamma\Phi_1 = k_1^2 < k^2 = \left(\frac{n\pi}{l}\right)^2 < k_2^2 = \gamma\Phi_2. \tag{A.65}$$

The Influence of Scale

This brings us to the important matter of the effect of scale: Note that the longest wavelength that can be driven unstable (giving the smallest possible wave number k) is that corresponding

to $n = 1$, that is $k = \pi/l$ (or $\lambda = 2l$). Thus if γ is small enough, such that $\gamma\Phi_2 < \pi/l$, the above inequality (A.65) will not even allow the $n = 1$ mode to form — there is not enough 'space' for even a single wavelength (where this wavelength is kinetically determined, as Φ_1 and Φ_2 depend on the kinetics). In this case, even though Re $\sigma(k^2) > 0$ for a range of k, none of these unstable k are allowed from the geometry; thus no mode will be driven unstable, and no spatial pattern will be generated.

What is the meaning of the condition 'if γ is small enough'? In nondimensionalizing the reaction-diffusion system to the general form (A.19)– (A.20), a typical length scale L was chosen for the system, to define the dimensionless parameter γ (for example, for the nondimensionalization of the Brusselator performed in section 3.2.3, the appropriate γ is $\gamma = L^2k_4/D_X$. In general, the γ obtained from such nondimensionalizations is proportional to the square of a typical length scale; that is, $\gamma^{1/2}$ is proportional to the linear size of a one-dimensional spatial domain, or γ is proportional to the area in two dimensions. Thus the inclusion of the term γ into the nondimensional equations is a convenient way of assessing the effects of a change in the domain size, merely by altering γ and noting the effect it has on the allowed patterns. Our discussions below on the effects of varying γ may thus be interpreted immediately as the consequence of *domain growth* on patterning (assuming that the parameters lie within the Turing space). Growth of the domain during patterning has obvious implications for biological development, so that the study of its effects is important for such applications, and some numerical simulations of growth have been presented by Arcuri and Murray [3] and Eilbeck [93] (the latter gives a bifurcation diagram of the different modes and amplitudes of the solutions possible for different parameters γ). Further studies on the response of reaction-diffusion structure on growth of the domain are given by Lacalli and Harrison [197], and especially the application to *Drosophila* compartmentalization by Kauffman *et al.* [180] (see section 4.3.3).

The result that no spatial patterning is possible for suffiently small γ, may thus be interpreted as the *nonexistence of spatial structure in a small domain*. This motivates the introduction of the concept of *critical domain size*, which is important in developmental biology and in spatially dependent ecological models. An alternative interpretation is obtained by noting that a small domain corresponds to strong diffusive effects, which act to wipe out spatial patterning — this accords with the nonexistence results for pattern formation obtained earlier, in section A.1.4.

The First Non-Trivial Pattern: Gradient Formation If γ increases sufficiently that $\gamma\Phi_1 < \pi/l < \gamma\Phi_2$, then the mode corresponding to $n = 1$ can be driven unstable; the corresponding first non-trivial pattern, predicted from linear theory and verified by, for example, singular perturbation analyses in the vicinity of bifurcation as well as numerical calculations, is the single *half-wave cosine mode*, proportional to $\cos(n\pi x/l)$. Note that this mode essentially represents a gradient, so that its formation represents a possible solution, other than simple diffusion [68], to the problem of gradient formation in development; potential biological implications of this are discussed at some depth in section 4.2.2.

Note that with the selection of the $n = 1$ mode, the pattern is not yet fully determined, as either the cosine function or its inversion (proportional to $-\cos(n\pi x/l)$) are still possible, resulting in two opposite potential polarities. Which one is chosen depends on any bias in the initial conditions; small perturbations such as occurring in any physical situation will nudge the solution into one or other of the possible patterns with equal probability. To insist on

one particular polarity, a specific asymmetrical disturbance or inhomogeneity has to be imposed which will break the $\pm$ symmetry. (This dependence of the final polarity on the initial conditions appears to pose a conceptual problem in development: the *bias* required for the reproducible orientation of patterns relative to previously formed structures could arise from the previous patterns; but then we appear no longer to have strict symmetry-breaking and self-organization. This point is discussed in more depth in the main text.)

The Outcome of Further Growth: Higher Modes and Finer Detail The effects of further growth are apparent by a similar analysis to the above: as γ, corresponding to scale, increases, this corresponds effectively to shifting the dispersion relation Re $\sigma(\mathrm{k}^2)$ to the right (see figure A.2), and higher wave numbers may become unstable. For example, since $\gamma \propto L^2$, doubling the linear domain size corresponds to quadrupling γ, so that, where previously the $n = 1$ mode was unstable, say, now the $n = 2$ mode becomes excited, according to the inequality (A.65). In general, the unstable wave numbers may be found from the integers n which satisfy (A.65); as γ increases, a greater range of integers n, and hence of wave numbers, will satisfy this inequality. Thus with successive domain growth, we expect increasingly finer detail and heterogeneity in the pattern to develop. Note, however, that patterns may also be lost on increasing size: if, say, for $\gamma = \gamma_1$ the $n = 1$ is unstable, then there may exist some γ with $\gamma_1 < \gamma < 4\gamma_1$ for which again no k values satisfy the inequality (A.65) — so domain size above the critical domain size (and parameters within the Turing space) is not yet a guarantor of spatial symmetry-breaking. This is a further demonstration of the intimate interplay between the geometric and kinetic constraints; the loss of structure intermediate between two modes was demonstrated for example in the analyses of Lara Ochoa and Murray [199], and Eilbeck [93].

Pattern Formation in Two Spatial Dimensions

The corresponding situation in two dimensions may be studied similarly: Consider (for analytical simplicity) a rectangular domain Ω defined by $0 \leq x \leq p$, $0 \leq y \leq q$. The spatial eigenvalue problem is still the problem of finding the eigenfunctions of $-\nabla^2$, but now on a two-dimensional domain, with zero flux boundary conditions $(\hat{n} \cdot \nabla)\phi = 0$ on $\partial\Omega$. The eigenfunctions in this case are

$$\phi(x, y) = \cos \frac{n\pi x}{p} \cos \frac{m\pi y}{q}, \quad \text{for integers } n,\ m, \tag{A.66}$$

with corresponding eigenvalues

$$k^2 = \pi^2 \left(\frac{n^2}{p^2} + \frac{m^2}{q^2} \right). \tag{A.67}$$

The rest of the analysis is as before, and yields the result that the unstable modes are those for which integers m and n satisfy

$$\gamma\Phi_1 = k_1^2 < k^2 = \pi^2 \left(\frac{n^2}{p^2} + \frac{m^2}{q^2} \right) < k_2^2 = \gamma\Phi_2. \tag{A.68}$$

In this case, the allowed patterns depend even more critically on the *domain geometry*, measured by length p and width q. For example, note that if the width is sufficiently narrow (that is, q is small enough) then even the first mode with $m = 1$ may lie outside the unstable

range, so that only modes with nonzero n are excited, and we obtain quasi-one-dimensional patterns, or *stripes*. As the width q increases, genuine two-dimensional patterns with $n \neq 0$ and $m \neq 0$ may become unstable, since then $\pi^2 \left(\frac{n^2}{p^2} + \frac{m^2}{q^2}\right)$ lies in the range of unstable wave numbers. Similarly, an increase in the size of the domain, conveniently measured by γ, could cause a pattern of stripes to break up into *spots*, as nontrivial modes become excited in both directions.

In general, linear theory predicts that for two-dimensional rectangular domains, cosine functions in each direction are obtained; other regular planar tesselation patterns may also be obtained, including solutions with hexagonal, square or rhombic symmetry. The exact resultant pattern of 'stripes' and 'spots' depends on the nonlinear effects, which distort the solution from the geometrically symmetrical linear predictions, as well as on the initial conditions. It is clear that the corresponding situation in three dimensions is even more intricate.

A.4.3 Some Comments on Nonlinear Effects

As a consequence of the above-mentioned scale and geometry constraints, there is a discrete range of wave numbers which may become unstable; the dispersion relation aids in the prediction of the unstable modes and their relative rates of growth, and hence provides some indication of the expected final pattern. These considerations are however ultimately based on linearization (which yields essentially equivalent results for all reaction-diffusion systems, as discussed above). To discriminate between different systems, the dimensions of the Turing space and the range of unstable wave numbers may be evaluated for each set of model equations individually, which already gives an indication that there are different classes of systems which respond differently, for example, to changes in size [197].

For an accurate indication of the behaviour of the system well beyond bifurcation, it is however necessary to solve the full nonlinear equations numerically, for instance with random perturbations about the steady state as initial conditions. Such studies, of which many have been performed (see for example [3, 93, 194], and any of the references given in the applications of reaction-diffusion systems in chapter 4), indicate that linear theory is generally a good predictor of the final pattern only in the cases where one or two wave numbers become unstable, and particularly if the unstable modes have large wavelengths, that is small wave numbers. Once we proceed to the realm of larger k values or several unstable modes, the predictions are less reliable, and even less so for two-dimensional patterns; the nonlinear coupling interaction between the modes then becomes crucially important, and different systems respond differently to varying domain growth and superposition of modes. This agrees with our previous remarks that linear predictions and singular perturbation analyses are less valid away from bifurcation boundaries (see section A.3.2).

Pattern Initiation In the strongly nonlinear domain, one critical influence on the resultant pattern is the mode of initiation, or the initial conditions. This is because the self-organizing dynamics typically embodies the possibility of multiple stationary states and non-uniqueness. Thus one finds, for example, that a different effect may be obtained depending on whether the instability is initiated by random perturbations in a domain that is already well above the critical domain size, so that multiple peaks form simultaneously (in which case one would expect

the wavelength of the pattern to correspond to that of the fastest growing mode), or whether patterns progress through the $n = 1$ gradient pattern to higher wave numbers and superposition of modes as a result of growth of the domain. The pattern could, alternatively, be initiated through a travelling wave originating from one end, so that the wavelength of the pattern is determined by that of the initiating wave. Lastly, in a situation of biological development and growth chemical or biochemical changes might result in the pattern at a particular time being 'frozen in', or fixed irreversibly, so that the wavelength of the final pattern is that corresponding to the time of pattern fixing, rather than to the final domain size. A further discussion of the effects of pattern initiation is given in section 4.3.1.

Spots and Stripes As we have already noted, linear theory is essentially limited in its ability to provide a basis for discriminating between different reaction-diffusion mechanisms, as the linearization of all nonlinear equations is basically equivalent. To differentiate between different models, numerical analyses have to be performed to provide a general indication of their patterning behaviours. A factor which is becoming increasingly important in distinguishing the specific properties of various models and their correspondence with experiment is the question of 'spots *versus* stripes' [150]: in two dimensions, the nonlinear coupling between modes causes some systems to display a predeliction towards more isolated peaks, while others are more inclined to 'join up' their peaks to produce more striped patterns. Which behaviour is favoured depends on the domain geometry (for instance the ratio of length to width — see above) but also on the specific form of the nonlinear interactions. A few reaction-diffusion systems have been shown to have a preference for stripe formation — see the discussions in sections 4.3.1 and 4.3.3 [235, 195, 211]; this feature is important to establish the plausibility of a model for particular applications (for example for stripe formation in *Drosophila* — see section 4.3.3).

The Two 'Families' of Reaction-Diffusion Systems

Numerical investigations of particular reaction-diffusion models that have been proposed for applications have revealed two 'families' of models that display contrasted behaviour. The Gierer-Meinhardt model [114] (see sections 4.2.2 and 4.3.1) and the Brusselator [310] (see section 3.2.3) are motivated and discussed in some depth in the main text; they provide the paradigmatic examples of two fundamentally different types of behaviour, which Harrison has described as 'headstrong' and 'adaptable', respectively (see [150]). Basically, the difference between the models is in how readily the patterns adjust to variations in system size, and regulate the interaction between the intrinsic kinetics and the domain geometry.

The *Brusselator* model, introduced initially as a simple chemical realization of reaction-diffusion kinetics (see section 3.2.3), and an example of a 'positive feedback' model in the sense of section 3.2.2 [87, 150], produces patterns that readily adjust to the system size by appearance, disappearance and movement of peaks, to achieve the optimum steady state pattern. The intrinsic ('chemical') wavelength is paramount, so that the pattern is dominated by the *kinetics*; the long-range correlations characteristic of self-organizing systems (see section 3.1.2) are particularly pronounced here, so that on perturbations or changes in the system size, the pattern responds automatically so as to maintain its intrinsic wavelength. Thus domain growth is liable to lead to a well-defined, discrete sequence of patterns, as the pattern 'adapts' to the domain size; such behaviour has been utilized in applications (for example [180]). Computations have

furthermore shown that the Brusselator readily converts random initial conditions into a regular hexagonal array of peaks with time [194], providing further evidence for the primacy of the intrinsic wavelength in determining peak spacing.

The *Gierer-Meinhardt* model, on the other hand, which was developed on the basis of considerations of activation and inhibition (see sections 3.2.2 and 4.2.2), is far more robust with respect to size variations. Once a pattern is established, it is stable, and long-range communication along the system seems to be lost. Thus, for instance, randomly distributed peaks established by chemical 'noise' on a two-dimensional domain are maintained; in contrast to Brusselator patterns, they display no regularity, only second-order statistics, in their distribution, and show little or no tendency to adjust their spacing to the preferred chemical wavelength [112, 194, 229]. Similarly, the initially-established monotonic gradient in a one-dimensional domain is robust to domain growth over a far greater range of system size than would be expected from linear theory [197]. Such behaviour motivates the description of the Gierer-Meinhardt model as 'headstrong', and unwilling to respond to changes in the system parameters.

Hints of these contrasting behaviours are given by linear theory [197], but ultimately it is the nonlinear interactions which determine such essential features of the reaction-diffusion system behaviour as robustness of pattern, and the ability to 'measure' domain size; hence numerical investigations (see appendix A.5) are essential to the classification of and discrimination between different reaction-diffusion mechanisms. A full characterization and appreciation of the differences between reaction-diffusion systems awaits further study; for an introduction to this problem, see Harrison [150].

The Relation between the Two Interacting Species We have, throughout our considerations in this section A.4 of the patterns that may be formed, neglected to consider that there are in any of the reaction-diffusion systems under study, *two* state variables which have inhomogeneous solutions. How are their patterns related? Firstly, in a developmental situation, one might generally expect the system to respond to the concentration of only one chemical species, or morphogen, in which case the other, although crucial for the *establishment* of pattern, may essentially be ignored when considering the isomorphism between the pattern and the final biological structure. But the real justification for being able to consider only one species is that the kinetics of the interaction between the two chemical species (or nonlinear coupling between other state variables described by a reaction-diffusion system) prescribes that the peaks and troughs of the final patterns of the two species are essentially similar, so that the concentration distribution pattern of one may be derived from the other.

There are two qualifications to this comment: firstly, one of the species (for example, an 'inhibitor' — see section 3.2.2) diffuses much faster than the other, the 'activator'; a necessary condition for spatial instability was found to be $d > 1$. This means that the activator peaks (those described by u in our general two-species formulation of appendix A.2) are much sharper than those of the inhibitor, v, as the latter are more smoothed out by diffusion. The second proviso is that the gradients in the two species may either be parallel (with overlapping peaks — the 'activator-inhibitor' models of section 3.2.2, of which the Gierer-Meinhardt model is an example) or anti-parallel, with peaks in the one pattern corresponding to troughs in the other (this corresponds to 'positive feedback' models, and is exemplified by the Brusselator). But essentially, taking due account of these qualifications, all our previous discussions regarding the

'pattern' of one species apply equally well to the other species participating in the reaction-diffusion system.

A.4.4 Some Results on the Diversity of Reaction-Diffusion-Generated Structures

We have considered various aspects of the types of patterns that may be formed by reaction-diffusion systems, at least in simple domains; and evaluated results concerning mode selection and pattern initiation, with reference to the dispersion relation. (One should note that the results and techniques discussed above hold generally for self-organizing pattern formation mechanisms with a dynamical systems formulation that have been proposed for development, not just for reaction-diffusion systems; see the book by Murray [251] for a more detailed discussion.) From the aforegoing, it appears that the diversity of available pattern-forming possibilities is somewhat limited. Indeed, we are restricted to a linear superposition of a finite number of eigenfunctions of the Laplacian on the domain of interest (as instability occurs for a finite range of the discrete ks), and to nonlinear 'modifications' or 'distortions' thereof, which may be expected, at least near bifurcation and for a small number of unstable modes, not to introduce too many novel features into the symmetries and patterns generated by reaction-diffusion systems.

Thus for one-dimensional systems, or rectangular domains (with zero flux boundary conditions) we obtain cosinusoidal modes; in circular domains the possibilities are Bessel functions; elliptical domains yield Mathieu functions, spherical regions, spherical harmonics and spherical Bessel functions, and so on. Within the ranges that reaction-diffusion mechanisms have been studied analytically and numerically (which involve mainly small k and few unstable wave numbers), the potential appears predominantly to be for the creation of periodic and quasi-periodic patterns. At any rate, with the limited eigenfunction 'building blocks' defined by the domain Ω available to create patterns, by no means *any* physically observed patterns seem to be potential reaction-diffusion concentration patterns.

Attempts have been made by some workers to place the above exploratory considerations on the types of possible reaction-diffusion patterns on a more rigorous footing.

A Measure of the 'Heterogeneity' of Spatial Pattern

As a measure for the potential for spatial pattern formation and possible detail, Berding [30] has introduced a 'heterogeneity' functional for the spatial patterns generated by reaction-diffusion systems with zero flux boundary conditions. We present his definition in one spatial dimension, with $x \in [0,1]$, and with the measure of domain size contained in the parameter γ. We assume that the reaction-diffusion system (A.61) evolves to the (possibly spatially heterogeneous) time-asymptotic stationary solutions $u = U(x)$, $v = V(x)$, which satisfy

$$U'' + \gamma f(U,V) = 0, \quad d\,V'' + \gamma g(U,V) = 0, \tag{A.69}$$

with boundary conditions

$$U'(0) = U'(1) = V'(0) = V'(1) = 0$$

(where $U' \equiv dU/dx$, and similarly for V'). Then the non-negative heterogeneity function is defined by

$$H = \int_0^1 (U'^2 + V'^2)dx \geq 0; \tag{A.70}$$

on integrating by parts, and using the defining equations for U'' and V'' and the zero flux boundary conditions, this becomes

$$H = \frac{\gamma}{d} \int_0^1 (dUf(U,V) + Vg(U,V))dx. \tag{A.71}$$

The plausibility of this H as a measure of heterogeneity is seen from its definition (A.70), which indicates that H increases as the pattern becomes more detailed and the slopes U' and V' become correspondingly steeper. If there is no spatial patterning, U and V are constant, uniform steady state solutions of $f(U,V) = g(U,V) = 0$, so that $H = 0$ as expected (no patterns mean $U' \equiv 0$, $V' \equiv 0$). Note that the scale parameter γ appears in the expression (A.71) for H. This corresponds to our previous recognition of the influence of scale: if γ increases, indicating a growing domain, then there is more heterogeneity, as higher modes may be driven unstable; this agrees with the increase in H predicted from (A.71). The main concern of Berding [30] was the demonstration of the utility of this heterogeneity function as a means of comparing theoretical predictions with experimental observations, rather than the derivation of any limitations on the possible values H could attain for any given system.

Bounds on the 'Complexity' of Reaction-Diffusion Systems

A more abstract study of the potential heterogeneity and level of detail possible in reaction-diffusion systems has been performed by Kopell and Ruelle [192], who have provided bounds on the 'complexity' of general reaction-diffusion systems, under the mild assumption (made in all realistic applications of reaction-diffusion kinetics) of compactness, that is that the equation system admits a bounded invariant region, as defined above (definition (A.4)). They have introduced a measure for temporal complexity h, which may be interpreted as an estimate for the (instantaneous) rate of growth of the unstable modes of the system, and a spatial complexity measure d, which may be taken as an upper bound for the number of unstable and neutrally stable modes. The asymptotic analogues of these may be viewed as bounds for the topological entropy and the Hausdorff dimension of the attracting set, respectively.

The major result of the analysis of Kopell and Ruelle [192] was the demonstration that these notions of 'complexity' are bounded, and the estimation of these bounds. These bounds may be regarded as furnishing limits on the density of subpatterns or on the magnitude of permitted wave numbers at any time, so that wavelengths, for example, do not become infinitely small (as they might in the absence of diffusion, when there are no restrictions on spatial scales). These results, which extend those we have already encountered in section A.1.4 on the nonexistence of spatial structure for reaction-diffusion equations with homogeneous Neumann boundary conditions on domains that are sufficiently small (the effect of a small domain is *equivalent* to that of large diffusion coefficients — see [40]), indicate that there is a *limit to the fineness of detail* that may be specified by means of reaction-diffusion mechanisms.

Summary: The Value of the Analytical Approach

The discussions of this section and the previous parts of this appendix, together with the two attempts just considered at characterizing the complexity of structure attainable through reaction-diffusion mechanisms [30, 192], thus give an indication of the patterns and structures that may or may not arise from reaction-diffusion-based mechanisms for spatial symmetry-breaking and self-organization. We shall in due course (see chapter 5 and appendix C) encounter other mathematical formulations for models of spontaneous pattern formation; analytical treatments of these would utilize analogous techniques, adapted to the class of equations under study, and might produce essentially similar results.

The mathematical analyses play an important role in indicating what patterns are possible or likely, hence confirming whether or not a proposed model is at all plausible in accounting for the origin of the patterns in the biological system under consideration. Especially in view of the fact that different models may frequently account for similar patterns (particularly if they are different manifestations of lateral inhibition models [287]), the analyses can however provide little more than corroborative evidence and plausibility arguments for a model. Ultimately, the application of these analytical results to a realistic situation must take considerations other than purely mathematical ones into account, such as the underlying biochemical or cellular interactions.

Perhaps the most important contribution the mathematical analysis has made is in rigorously demonstrating the possibility of spontaneous symmetry-breaking by bifurcation from a homogeneous steady state. Thus the concept of self-organization, which plays such a fundamental role in pattern formation and morphogenesis, as evidenced throughout this thesis (see especially chapters 4 and 5), is well-established.

A.5 Numerical Methods

We have considered in some depth the types of possible solution behaviour displayed by reaction-diffusion equations, as examples of self-organizing systems; in particular, our interest has focussed on the creation of inhomogeneous stationary spatial patterns from an initially uniform steady state. In the course of these analyses, it has become clear that everywhere except before and very near the point of bifurcation of the nontrivial patterned solution from the homogeneous steady state, analytical methods are extremely limited in their ability to predict anything more than coarse approximations to the solution behaviour, owing to the fundamental nonlinearities in the reaction-diffusion equations under consideration. In order to gain a further understanding of the actual solution behaviour, it is useful and frequently necessary to resort to **numerical methods**. The intention of this brief section is to provide essentially a mere mention of the diverse of numerical techniques that have been employed in the study of reaction-diffusion equations.

In contrast to the situation for systems of ordinary differential equations, where Runge-Kutta methods and their generalizations are sufficient in principle for their numerical solution, there are no general techniques applicable to partial differential equations, especially not for systems of nonlinear (parabolic) partial differential equations such as reaction-diffusion systems. Consequently, various authors, in their studies of reaction-diffusion systems (generally motivated by

considerations in developmental biology), have worked on the development of efficient numerical algorithms for the solution of reaction-diffusion and associated equations.

What follows below is not a general theory of numerical analysis of such systems, nor a detailed discussion or advocacy of any one of them, but rather a *brief indication* of the range and diversity of some of the numerical methods that have been used. The reader is referred to the original papers and in particular to the references therein for a detailed discussion of the methods mentioned here.

Finite Difference Methods

The simplest possible method for the numerical solution of parabolic partial differential equations is a fully explicit *finite difference method*, by which the solution is calculated at each time step solely on the basis of the previous one. Such a method is only stable under quite restrictive conditions for the simplest parabolic equation, the diffusion (or heat) equation (4.2) (see section 4.2.1), and should thus not be expected to be stable or particularly accurate for the more complicated, nonlinear reaction-diffusion systems. Nevertheless, such schemes have been utilized, notably by Meinhardt (he reproduces computer programs applying such calculations in [229, 237]).

Beyond the fully explicit method, perhaps the most elementary scheme that has been employed for the study of reaction-diffusion systems [53, 91] is known as the Hopscotch method [132, 133, 134]. This is a fairly fast second-order partial differential equation solver, easy to program, which forgoes high accuracy but enables the indication of qualitative features of the system. Each time step is accomplished in two passes over the spatial mesh, considering alternate grid points each time, to give an essentially explicit scheme. Its accuracy and stability is, as a result, not clear.

An alternative and more well-established class of methods is based on the method of lines, using again standard finite differences to discretize the partial differential equation in space, and solving the resultant (stiff) system of ordinary differential equations in time by using iterated Runge-Kutta methods modified to deal with a large and stiff system. Thus, for example, the NAG library implementation of Gear's variable-order, variable-step algorithm has been used [3], as have other purpose-made partial differential equation solvers based on similar principles [306].

For the numerical solution of reaction-diffusion equations in three space dimensions, Hunding has worked on similar methods, except that for n mesh points in each direction, the number of equations goes as n^3, increasing extremely rapidly with finer spatial resolution. Thus he has introduced methods adapted to the solution of the resulting stiff systems, and taking into account the sparsity of the coefficient matrix, which speed up computation time considerably over standard, non-stiff Runge-Kutta techniques; these enable the treatment of the analytically and numerically complex and time-expensive, but physically important, three-dimensional reaction-diffusion systems [165, 170].

Finite Element Methods

An alternative to the finite difference methods above is the class of *finite element methods*, where, rather than carrying out the spatial approximation by calculations at a finite number of

discrete grid points, the solution is expressed as a truncated series in finite-dimensional function space (with a discretization in time using finite differences). The standard finite element analysis of a reaction-diffusion system has been carried out, for example, by Kernevez and coworkers, who also describe the method used in their works — see for example [47, 188, 190]. Essentially, the spatial domain Ω (in two dimensions) is divided into M elements Ω_m, on each of which the function $\mathbf{u}(x, y)$ is approximated by a polynomial expression (the elements they use are eight-node quadrilaterals). Using this method, the reaction-diffusion system on fairly nonuniform domain shapes, approximating for example the actual shapes of *Drosophila* wing disks, could be simulated without significant extra effort. Murray [246, 247, 248] used the method (and code) of the above authors to solve his model reaction-diffusion equations on different domain shapes in his study of pattern formation on animal coats.

An alternative approach to the use of finite elements is one using Galerkin finite element methods, used for example for a numerical study [219] of reaction-diffusion equations arising in the Belousov-Zhabotinskii reaction (see appendix B) — here a hybrid scheme, comprising a Petrov-Galerkin finite element procedure in space, followed by a Crank-Nicholson finite difference discretization in time, was employed.

Pseudo-Spectral Methods

The last method to be mentioned here, which has shown some promise for the study of reaction-diffusion equations, is a collocation approach, or *pseudo-spectral method* [92] (also used in [215]). The approach is based on the synergetic concept of 'order parameters': the assumption is that a large number of degrees of freedom can be described by a limited number of collective modes, corresponding to the order parameters (coefficients of excited modes) and those damped modes (with 'slaved variables' as coefficients) which are strongly coupled to the excited eigenfunctions through the nonlinearities (see the discussion in sections 3.1.4 and A.3, and also Haken [141]). The solution function is written in terms of the eigenfunctions ϕ_j of the linearized operator, truncating the infinite series expansion at some (arbitrary, but well-chosen) cutoff point to include only those modes which contribute significantly to the final solution — thus one approximates the solution (for example in one dimension) by

$$\mathbf{u}(x,t) \approx \sum_{j=1}^{N} c_j(t)\phi_j(x); \tag{A.72}$$

substitutes this form into the reaction-diffusion system, and solves for the evolution of the amplitudes $c_j(y)$ at the collocation points $x_1, x_2, \ldots, x_N$, which gives N coupled nonlinear evolution equations for the N amplitudes; these may be solved straightforwardly using standard ordinary differential equation solvers. This method thus proceeds in the spirit of our discussion of bifurcation theory and synergetics, and the solution behaviour these techniques predict; in fact it requires fewer spatial grid points than finite difference methods, while permitting a larger time step, and thus holds promise for future applications. A superficial study such as the present one cannot assess this in any detail, however.

This section was intended to be merely a cursory glance at some of the numerical methods that have been applied to the study of reaction-diffusion and allied parabolic partial differential equation systems. Clearly, in contrast to the situation for ordinary differential equations, no

'black box' techniques exist, and active research on the most efficient and accurate numerical techniques is proceeding; as the above-mentioned methods all have their own advantages and disadvantages in terms of accuracy, permitted time step magnitudes, required computing time and memory, and so on. The aim here was to draw attention to this situation of the existence of a diversity of techniques, in order to indicate that the numerical solution of model equations, as referred to in the main text, is not mysterious or unintelligible, but is by no means a straightforward and trivial problem.

Appendix B

The Belousov-Zhabotinskii Reaction

Our study of reaction-diffusion equations, limit cycles and other purported self-organizing behaviour may appear to be merely a construct of the mathematics (see appendix A, and chapter 3). That such spatial and temporal pattern formation may be observed experimentally is best demonstrated by a fascinating, and much-studied, chemical reaction known as the Belousov-Zhabotinskii reaction, which under certain conditions of nonequilibrium displays an entire spectrum of unexpected, compelling and also visually pleasing behaviours; it is generally considered the prototype chemical oscillator, although similar systems have since been discovered and studied.

There is nothing particularly special about the reactants in the Belousov-Zhabotinskii reaction (which is commonly abbreviated as the BZ reaction). The basic mechanism consists of the oxidation of malonic acid, $CH_2(COOH)_2$, in an acid medium, by bromate ions (using for example potassium bromate $KBrO_3$), and catalyzed by ceric-cerous (Ce^{4+}/Ce^{3+}) ion couple (added in the form of cerous sulphate $Ce(SO_4)_3$, or some other cerium salt). Under typical experimental conditions, the reagents are dissolved in sulphuric acid, H_2SO_4, at room temperature, 25°C. In following the dynamics of the reaction, the observables of interest are, for example, the concentrations of the cerium ions.

The evolution of the system may be followed through sophisticated means of observation such as specific electrodes, or spectroscopic measurements of optical absorption caused by one particular substance; more directly, however, observations may be with the naked eye, if other metal ion catalysts such as iron, Fe^{2+} and Fe^{3+}, and appropriate coloured indicator dyes, such as phenanthroline or ferroin, are added. Ferroin, for example, gives a red colour when there is an excess of ions of Fe^{2+}, and turns blue under conditions of excess Fe^{3+}.

This reaction, based on reagents readily available in any chemical laboratory, is of interest due to the range of its possible behaviours, which will be discussed in some depth below. Summarizing briefly, the reaction supports an abrupt halogen-based oxidation-reduction reaction, in consequence of which under appropriate conditions sustained periodic oscillations in the chemical concentrations are observed in the well-stirred solution, with a period of the order of one minute. If the reaction mixture is not well stirred, concentric and spiral wave patterns may be produced, as well as scroll waves in three dimensions. The presence of stationary spatial patterns (Turing structures [354]) has also been demonstrated in chemical reaction systems, though not directly in the BZ reaction.

As this is the chemical oscillator (and indeed system exhibiting dissipative structure in general) which is most-studied and best-understood — most of the observed behaviour of the system has been accounted for qualitatively and even quantitatively to a fair degree of accuracy by an analysis of the kinetic equations that will be presented below — a fairly comprehensive literature has accumulated on the BZ reaction. For an early general introduction see [366] (also see Winfree's book [367] for a good general overview). Brief introductions to the main features may be found in [10, 276, 277] and to the analysis in [245, 251]. More detailed and comprehensive discussions are available particularly in Tyson [355] and in the book of articles edited by Field and Burger, [98]. The discussion below will give only a brief introduction to this clear example of self-organization — the reader is referred to the above texts and to the extensive literature cited therein for a more detailed analysis.

B.1 Mechanism of the BZ Reaction

Outline of the BZ mechanism We briefly discuss the key steps in the reaction, and introduce the Field-Noyes model [100] which quantitatively mimics the actual chemical reactions. We consider first the reaction occurring in a well-stirred medium; under these conditions, sustained oscillations in the chemical concentrations occur for certain ranges of initial composition of the mixture; so that the system behaves essentially as a *chemical 'clock'*, measuring time through internally generated dynamics. The temporal evolution of the concentrations of Br^- and the ratio $[Ce^{4+}]/[Ce^{3+}]$ is plotted in figure B.1, after potentiometric traces due to Field, Kőrös and Noyes (1972) [99].

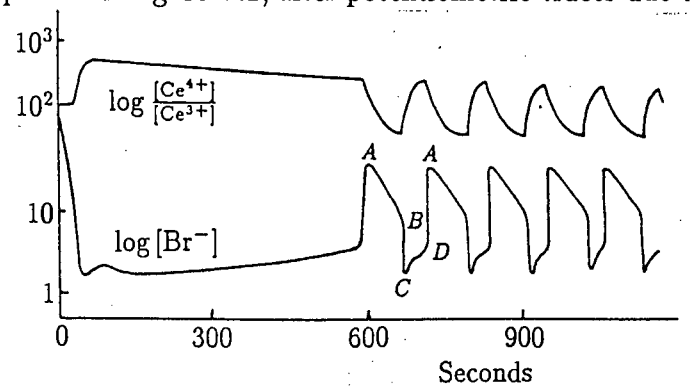

Figure B.1: Experimentally measured periodic temporal variation in the ratio of the cerium metal ion concentrations $[Ce^{4+}]/[Ce^{3+}]$ and in the bromide ion concentration $[Br^-]$ in the Belousov-Zhabotinskii reaction. (Reproduced from [251, p.180])

After an initial 'incubation time', the period of the oscillations is of the order of minutes (about two minutes according to the results of figure B.1); this period is asymptotically stable to disturbances, unlike for example the period of oscillations in a conservative system such as the pendulum. Under closed system conditions, the oscillations eventually die out, as the overall reaction is irreversible, and for a system closed to mass transfer, the reagents will eventually be used up; the life time of the phenomenon depends on the initial concentrations, but is of the order of an hour. Eventually, a state of thermodynamic equilibrium is reached, in which temporal oscillatory behaviour is ruled out. The reaction has, however, also been performed under open system conditions, with a constant flux of reagents; in these circumstances, the

oscillations may be sustained indefinitely. The sharpness, stability and reproducibility of the oscillations indicates that the oscillations belong to the class of *chemical dissipative structures*, as is substantiated by the mathematical model of the reaction mechanism to be discussed below.

As may be deduced from figure B.1, the reaction may be separated into two parts, say I and II; which one is dominant at any time depends on the bromide concentration $[Br^-]$.

Part I : When $[Br^-]$ is sufficiently high, I is dominant, and Br^- is consumed; that is, we move along AB. As the bromide concentration decreases further, it falls below a critical value at B, and then drops quickly to a low level, at C in figure B.1. At this stage process II takes over.

Part II : The Ce^{3+} ions are oxidised to Ce^{4+} towards the end of the I process; however, in the II process Ce^{4+} reacts to produce Br^- again, and reverts to the Ce^{3+} state. The bromide concentration increases, at first slowly and then more rapidly, until it is high enough, when process I again becomes dominant.

The FKN Mechanism

The mechanism of the reaction is more or less completely understood; there is a fairly large number of stable and unstable intermediates, so that at least eighteen elementary reactions have been identified (in the early studies; recent work reveals many more, and extensive mechanistic studies are continuing — see the articles by Field and Tyson in [98]). An introductory description of the major processes is given in [276], with a more detailed discussion given by Tyson [355]. The full system has been simulated on computer, but it is very complex, and thus opaque to any analytical understanding. Fortunately, by considering which processes are rate-limiting and important, it has been possible to reduce the reaction system to five key reactions, as suggested by Field and Noyes [100]. (Note that a different analysis is available, based on an alternative mechanism for the BZ reaction proposed by Zhabotinskii and co-workers; the Zhabotinskii-Zaikin-Korzukhin-Kreitser model and its theoretical implications are briefly discussed in [355].) The five reactions (not all elementary) considered by Field, Kőrös and Noyes [99] to be most important for the kinetics (the FKN mechanism, which has essentially stood the test of time — see [98]) are:

$$Br^- + BrO_3^- + 2H^+ \xrightarrow{k_1} HBrO_2 + HOBr \tag{B.1}$$

$$HBrO_2 + Br^- + H^+ \xrightarrow{k_2} 2HOBr \tag{B.2}$$

$$2Ce^{3+} + BrO_3^- + HBrO_2 + 3H^+ \xrightarrow{k_3} 2Ce^{4+} + 2HBrO_2 + H_2O \tag{B.3}$$

$$2HBrO_2 \xrightarrow{k_4} HOBr + BrO_3^- + H^+ \tag{B.4}$$

$$4Ce^{4+} + BrCH(COOH)_2 + 2H_2O \xrightarrow{k_5} 4Ce^{3+} + HCOOH + 2CO_2 + 5H^+ + Br^- \tag{B.5}$$

The above reactions summarize the main features of the BZ system. Roughly, reactions (B.1) to (B.3) correspond to part I of the overall mechanism, with the depletion of Br^-, which is in competition with BrO_3^- for the $HBrO_2$; whereas the last two reactions, (B.4) and (B.5), refer to part II, where Br^- is regenerated again. This process of bromide depletion and regeneration can, in principle, occur indefinitely, which leads to the observed oscillations. The conditions for thermodynamic nonequilibrium that are necessary for dissipative structures to be observed may be traced back especially to reaction (B.3), the autocatalytic production of $HBrO_2$.

B.1.1 The Field-Noyes Model

Field and Noyes (1974) [100] proposed a kinetic model for the BZ reaction, based on the above mechanism; essentially, they isolated three key substances whose kinetic behaviour could account for the observed oscillations and other phenomena. The key chemical elements considered in the above reactions are

$$X = HBrO_2, \quad Y = Br^-, \quad Z = Ce^{4+},$$

$$A = BrO_3^-, \quad B = (\text{bromomalonic acid} + \text{malonic acid}), \quad P = HOBr;$$

and the model reactions may be approximated by the sequence

$$A + Y \xrightarrow{k_1} X + P, \tag{B.6}$$

$$X + Y \xrightarrow{k_2} 2P, \tag{B.7}$$

$$A + X \xrightarrow{k_3} 2X + 2Z, \tag{B.8}$$

$$2X \xrightarrow{k_4} A + P, \tag{B.9}$$

$$B + Z \xrightarrow{k_5} fY, \tag{B.10}$$

where f is a stoichiometric factor, usually taken to be 0.5, and the rate constants $k_1, \ldots, k_5$ are considered to be known. As indicated above, the first two or three reactions are roughly equivalent to process I, while the rest relate approximately to process II. Note that certain assumptions, about excess concentrations of some chemical and relative insignificance of others, have been incorporated into this model. As the reactions are approximations to the detailed mechanism, there has been some debate about the appropriate rate constants to be used; a recent set of values [183] is

$$k_1 = 2M^{-3}s^{-1}[H^+]^2, \quad k_2 = 10^6 M^{-2}s^{-1}[H^+],$$

$$k_3 = 40M^{-2}s^{-1}[H^+], \quad k_4 = 2 \times 10^3 M^{-1}s^{-1}, \quad k_5 = 0.4M^{-1}s^{-1}.$$

From the above system, it is possible to derive kinetic equations; in order to do so, it is reasonable to take the bromate ion concentration $[A]$ and the concentrations of malonic acid and bromomalonic acid, [MA] and [BrMA], to be constant (they do not change significantly over times of the order of minutes), while the concentration $[P]$ is of no relevance here, as it does not affect the rate laws if the reactions are taken to be irreversible. Thus we may derive the kinetic (rate) equations in the form of three differential equations:

$$\frac{du}{dt} = k_1 av - k_2 uv + k_3 au - k_4 u^2 \tag{B.11}$$

$$\frac{dv}{dt} = -k_1 av - k_2 uv + f k_5 w \tag{B.12}$$

$$\frac{dw}{dt} = 2 k_3 au - k_5 w \tag{B.13}$$

where $a = [A] = [BrO_3^-] \approx$ constant, $b = [B] = [MA] + [BrMA] \approx$ constant, $u = [X] = [HBrO_2]$, $v = [Y] = [Br^-]$, $w = [Z] = [Ce^{4+}]$.

This system is often referred to as the 'Oregonator' (by analogy with the 'Brusselator' — see section 3.2.3) as it exhibits limit cycle oscillations and the research by Field *et al.* [99, 100] was done at the University of Oregon.

B.2 The Existence of Oscillations

An analysis of the above differential equation system reveals the experimentally observed oscillatory behaviour, in the mathematical form of a limit cycle (see appendix A.3.2), depending critically on certain parameter values, in particular f and k_5. A brief outline of the analysis is given below:

B.2.1 Nondimensionalization and Linear Stability Analysis

In order to proceed, it is best to nondimensionalize the system (see appendix A.4.1). Several such nondimensionalizations are possible, which may reveal or emphasize different features of the system; we follow the analysis of Murray [251, pp.182–190], and introduce new variables as suggested there. Then our system becomes

$$\varepsilon \frac{dx}{dt} = qy - xy + x(1 - x), \tag{B.14}$$

$$\delta \frac{dy}{dt} = -qy - xy + 2fx, \tag{B.15}$$

$$\frac{dz}{dt} = x - z. \tag{B.16}$$

With the above rate constants and the appropriate nondimensionalization, the model parameters $\varepsilon \approx 10^{-2}$, $\delta \approx 10^{-4}$ and $q \approx 2 \times 10^{-4}$ are 'small'.

Although the system (B.14)–(B.16) is third order, the linear stability analysis proceeds exactly as for the second order case. By setting the time derivatives equal to zero, the steady states may first be found; there are two non-negative steady states, with coordinates $(0, 0, 0)$ (the trivial steady state, with zero reactant concentrations) and

$$x_s = \tfrac{1}{2}(1 - q - 2f) + [(1 - q - 2f)^2 + 4q(1 + 2f)]^{1/2},$$

$$y_s = \frac{2fx_s}{q + x_s}, \quad z_s = x_s. \tag{B.17}$$

Linearizing about the zero solution, and finding the eigenvalues of the stability matrix A, we find that the trivial steady state is always linearly unstable; clearly, oscillations about the origin would also require some concentrations to become negative, which is nonphysical. For stable limit cycle solutions to exist, we thus need the nontrivial steady state (x_s, y_s, z_s) to be an unstable critical point.

Linear Stability Analysis We thus perform a linear stability analysis about this steady state, which gives, for the eigenvalues λ of the linearized matrix,

$$\lambda^3 + A\lambda^2 + B\lambda + C = 0, \tag{B.18}$$

where A, B and C are complicated functions of the parameters of the equations [251, p.184]. The nontrivial steady state is linearly stable if all the roots λ of this equation have negative real parts; necessary and sufficient conditions for this are obtained from the Routh-Hurwitz conditions (see, for example, [251, appendix 2]), as

$$A > 0, \quad C > 0, \quad AB - C > 0. \tag{B.19}$$

Now the analysis shows that for realistic (ie. positive) values for the parameters, A and C are both always positive, whereas B can be positive or negative; thus the condition for instability is the violation of the third inequality. After some algebra, the *instability condition* may be rewritten in the form

$$AB - C = \phi(\delta, f, \varepsilon) = \frac{N\delta^2 + M\delta + L}{\delta^2} < 0, \tag{B.20}$$

where L, M and N are functions of δ, f, q and ε.

The bifurcation or instability surface is given by $\phi(\delta, f, \varepsilon) = 0$; for given values of q, this places a restriction on the values of f and ε for which there are values of δ giving $\phi < 0$. Using the fact that ε is small, we may obtain two critical values of f, ${}_1f_c$ and ${}_2f_c$ for a given q, such that instability may occur for intermediate values of f [251, pp.185–186]:

$${}_1f_c < f < {}_2f_c \tag{B.21}$$

is the condition on f so that instability is possible. Approximations to the critical values of f may be obtained for small q; in this case the range of f for which the positive steady state is linearly unstable is

$$\frac{1}{4} \approx {}_1f_c < f < {}_2f_c \approx \frac{1+\sqrt{2}}{2}; \tag{B.22}$$

and for each ε, the stability bifurcation curve of δ against f is given by

$$\delta_c = -\frac{M}{2N} + \frac{[M^2 - 4LN]^{1/2}}{2N} \tag{B.23}$$

for each f in the unstable range. The result of this linear stability analysis is thus that for $0 < \delta < \delta_c$, the nontrivial steady state (x_s, y_s, z_s) is unstable to small disturbances, and we must consider global stability to determine the existence of limit cycle solutions.

B.2.2 Nonlocal Stability and Limit Cycle Oscillations

Coming to the behaviour of the system past instability, we have noted that there is a range of parameter values for which the positive steady state is unstable, and indeed this turns out to be by growing oscillations if (δ, f) are close to the bifurcation curve. The existence of periodic solutions (as opposed to those which diverge to infinity — these are unrealistic) requires the existence of a confined, or *invariant*, set, S say, as defined in appendix A, definition (A.4). For a system of ordinary differential equations, as above, such a set S may be found to satisfy the condition

$$\hat{n} \cdot \frac{d\mathbf{r}}{dt} < 0, \quad \text{for } \mathbf{r} \text{ on } S, \tag{B.24}$$

where $\hat{n}$ is the unit outward normal to S, and $\mathbf{r} = (x, y, z)$ is the solution vector to the system of differential equations, such that $d\mathbf{r}/dt$ may be found from (B.14)–(B.16).

Such a confined set S may be found; it is [251, pp.187–190]

$$S = \{(x, y, z) |\ q \leq x \leq 1,\ \frac{2fq}{q+1} \leq y \leq \frac{f}{q},\ q \leq z \leq 1\}. \tag{B.25}$$

It can be shown furthermore that any solution beginning in the positive octant $x > 0, y > 0, z > 0$ will eventually enter S [355, chapter III]. Thus all solutions are known to be bounded. In three or more dimensions, this in itself is not sufficient to guarantee the existence of periodic solutions in the presence of an unstable steady state, as it is in two dimensions, where we have the Poincaré-Bendixson theorem (with three equations it is possible to have chaotic solutions, as for the classical Lorenz system, which exhibits chaos in a system of three ordinary differential equations).

However, for the particular case of the BZ reaction, Hastings and Murray (1975) (see [355, chapter III]) have given a rigorous proof, using topological methods and the Brouwer fixed point theorem, showing that the FN model system (B.14)–(B.16) contains *at least one* finite-amplitude periodic trajectory. Thus in this case it is established that limit cycle solutions exist; in the case where the steady state is unstable, this confirms analytically the presence of *oscillations* in the BZ reaction.

Analysis and Features of the Oscillations

To obtain a better understanding of the character of the limit cycle oscillations, the periodic solutions may be evaluated numerically, or constructed analytically using bifurcation theory or asymptotic methods, to obtain estimates for the amplitude and period of the oscillations. For further analytical simplicity and tractability, the system may be treated as a relaxation oscillator: we note from figure B.1 that certain parts of the cycle (BC and AD) are traversed very rapidly compared to other parts. From a modelling point of view, this means that a small parameter must be present in the equations, to cause a rapid variation in the solutions. For example, with $\varepsilon \ll 1$ in equation (B.14), x may be considered to adjust instantaneously to the concentration of y (x varies on a time scale much smaller than times for changes of y and z), so that the equations may be reduced to a two-variable system for y and z, with $x = x(y)$ obtained from setting $\dot{x} = 0$ in (B.14). The resultant two-variable system may now be analysed using phase-plane methods, and using bifurcation theory or asymptotic techniques to derive first-order

approximations for the limit cycle amplitude and period (see for example [251, 355]). The results obtained compare well with the experimentally observed values.

For the situation discussed above, the system is excited autonomously from the unstable steady state to enter a stable limit cycle oscillation — this is 'soft self-excitation'. Note that the system can also exhibit 'hard self-excitation' — for example for $f > {}_2f_c$, the critical point is stable, but there is a small-amplitude *unstable* limit cycle, surrounded by a stable limit cycle of larger amplitude. This implied that there *is* in this case an orbitally stable limit cycle, that is the potential for persistent oscillations, but it requires a hard, or finite, perturbation from the linearly stable steady state to reach this periodic solution. In this case we say that the system displays *excitable kinetics*; this is possible over a range of values of f (outside the range given by (B.21)), but sometimes an induction period is required to attain the oscillatory trajectory, as is demonstrated also in figure B.1.

Relation to Self-Organization We may interpret this behaviour in the general context of self-organization: Our chemical system has a homogeneous steady state, the stability of which is lost for certain parameter ranges; alternatively, the trajectory may be excited into a different regime of phase space. Suddenly the equilibrium solution becomes periodic, and the system 'discovers' time in the phase of oscillation and the specific temporal order of the concentration patterns and maxima — thus there is a breaking of temporal symmetry. Even more fundamentally, the maintenance of sustained oscillatory behaviour encompassing the entire system implies that its different parts act in a concerted fashion by maintaining sharp phase relationships; otherwise destructive interference would wipe out the oscillatory behaviour. Thus we observe the emergence of *long-range correlations* [274]. In this sense, the Belousov-Zhabotinskii reaction provides fundamental evidence of self-organization and the emergence of dissipative structure.

Complex Temporal Behaviour

Under certain conditions, chemical systems such as the BZ system can display even more complex temporal behaviour. In particular, as alluded to above, a three-variable system may display chaotic behaviour, which has been observed in theoretical and experimental studies of the BZ reaction (see for example [20, 164]) — "the well-stirred Belousov-Zhabotinskii reaction readily produces chaotic oscillations". Whereas in fluid mechanics complex behaviour is invariably associated with spatial inhomogeneities, for example in the Bénard experiment, the fact that autocatalytic chemical reactions can amplify concentration perturbations means that even a well-stirred chemical system can display complex, self-organized behaviour in time.

All the behaviour has been considered so far in a spatially homogeneous, or well-stirred, reacting medium. If we proceed to study the unstirred case, where diffusion effects must be included, a new and surprising range of phenomena appears, indicating spatial as well as temporal self-organization.

B.3 Spatial Patterns in a Nonuniform System

Suppose that the BZ reaction is carried out without stirring, allowing for the possible development of spatial inhomogeneities. One can then observe regular spatio-temporal patterns in the form of propagating wave-fronts, in one, two and three dimensions.

B.3.1 One-dimensional Kinematic Waves

Consider firstly a one-dimensional situation, with the reaction carried out in a thin, long vertical tube which is left unstirred. Then, when an appropriate indicator dye is added, such as ferroin mentioned above (if the BZ oscillations involve an iron catalyst), horizontal bands of blue and red appear, corresponding to alternately high and low concentrations of the chemicals. These bands usually start to appear at the bottom of the cylinder and move slowly upwards with successive bands moving progressively more slowly. Eventually the cylinder is filled by these bands but with a non-uniform density, with the wave packing being denser closer to the bottom.

Unlike for the waves to be discussed later (section B.3.2), diffusion plays a negligible role in the formation and propagation of these wave-like structures. If a barrier impermeable to any of the chemicals were placed in the cylinder it would affect neither the wave propagation nor the density of bands; spatial transport processes are not involved in the generation of this spatial pattern. Thus we should rather refer to these formations as *kinematic waves*, or 'pseudo-waves', as there is nothing actually being transported. Rather, the concentration bands may be accounted for by considering each horizontal level of the BZ reagent to be an independent (self-excited) oscillator with its own phase and period T, which may be a function of position; different regions do not interact. If these independent oscillators are out of phase or have different frequencies then the spatial patterns will appear simply as a consequence of the spatial variation in the phase or frequency. Such phase or frequency gradients may be set up by a concentration gradient in one of the chemicals, or by temperature variation.

The propagation of such kinematic waves can be considered analytically, with the predicted velocity of the waves and the patterns that are formed giving good agreement with experiment: If $T(z)$, the period as a function of the vertical coordinate z, is monotonically increasing, then bands appear at the bottom of the cylinder and propagate upwards, with successive bands moving increasingly slowly, so that the velocity of the k-th wavefront is

$$v_k(z) = \frac{1}{kT'(z)}; \tag{B.26}$$

see the analysis in [251, pp.254–258]. Diffusion effects only become relevant after a long time, when the kinematic wave concentration pattern is almost stationary and the spatial inhomogeneities will begin to be smoothed out by diffusion.

This example indicates that spatial concentration patterns may be produced as a consequence of a spatial variation in the oscillator parameters, in any chemical or biological system which has the potential for spontaneous oscillatory or clock behaviour, a situation which is prevalent in nature. Spatial patterning need thus not necessarily be dependent on a reaction-diffusion or other spontaneous pattern-forming mechanism; this may have considerable implications for considerations of biological pattern formation mechanisms.

B.3.2 Waves in Two and Three Dimensions

When the BZ reaction takes place in an unstirred shallow layer (for example in a petri dish), two main different forms of wave patterns are possible: circular wave fronts, displaying a roughly circular symmetry about an axis perpendicular to the thin layer; and spiral fronts, rotating in space clockwise or anticlockwise. It is also possible to obtain more complicated multi-armed spirals. In the case of the patterns of concentric circles, frequently referred to as target patterns (for obvious reasons), there is a range in the spatial frequency (or wavelength) of the waves. On the other hand, the spiral waves all rotate at the same frequency and present to the viewer spirals of identical pitch. In three dimensions the topological strucure is even more remarkable, with spiralling and scroll-like waves being observed.

The wave-like phenomena described cursorily above are best studied in a non-oscillatory, excitable medium — that is, for f discussed above outside the range of values of self-excitation (equation (B.21)), so that the (spatially and temporally homogeneous) steady state is stable, but also so that a large enough perturbation can drive the system to a stable limit cycle, that is, an oscillatory solution. In the BZ reaction, once a localized region is excited, waves are driven through space by the autocatalytic production of bromous acid in the wavefront (equation (B.3)) and the diffusion of $HBrO_2$ to regions of low bromous acid concentration ahead of the wavefront, pushing these regions over the excitation threshold and triggering the autocatalytic reaction there. Behind the wave front the transition metal ion is oxidized (B.3), bromous acid is removed by disproportionation (B.4), and finally the transition metal ion is converted slowly back to the reduced state (the 'recovery' phase) (B.5).

Trigger Waves and Target Patterns

When the medium is carefully prepared, the system will remain for a long time in a reduced, stable state, but if perturbed sufficiently, a single circular wave of oxidation (Ce^{4+} concentration maxima) will propagate away from the point of perturbation until it collides with the boundary of the dish and disappears. The perturbation produces local concentrations different from those of the surrounding medium, and the effect of diffusion is to smooth out such concentration differences — this can initiate a solitary travelling wave in concentration maxima, known as a *trigger wave*.

Target Patterns If less care is taken in preparing the mixture, expanding concentric waves are emitted periodically from pacemakers distributed randomly in the mixtures. In this case, there are probably small inhomogeneities, such as dust particles, that perturb the reagent and push it locally into an oscillatory mode. Thus there are periodic concentration imbalances that, together with the effect of diffusion which acts to counterbalance these concentration variations, produce travelling waves trains of concentration maxima propagating outward from a centre.

Such a situation can occur both if the surrounding medium is quiescent, and if it is oscillating — in both cases, the local inhomogeneity acts as a *pacemaker*, with a local oscillation period T different from the period T_0 of the bulk medium (which could be infinite for a quiescent medium, at the stable steady state), and so a wave of excitation is produced every T seconds. These target patterns (as well as the scroll waves mentioned above and discussed later) are blocked by impermeable barriers, indicating that they depend on diffusion. The spatial frequency of the

circular waves (which all have the same wave speed, characteristic of the medium) depends on the period of the localized oscillation T, which is determined by the exact chemical nature of the inhomogeneity; thus there is variation in these frequencies due to the random nature of the localized pacemakers. The target patterns are a manifestation of spatial symmetry-breaking — the system is no longer invariant to translation along a particular direction in space, analogous to the Bénard system.

Modelling of Trigger Waves Mathematically, it has been possible to model trigger waves (and hence target patterns) as a *threshold phenomenon*, on the assumption that the wavefront is dominated by Part I of the BZ reaction mechanism (see for example [251, pp.322–328] for a discussion); which implies in particular that the cerium is in the Ce^{3+} state, ie. $w = [Z] = [Ce^{4+}] = 0$. Thus we can reduce the reaction equations to a two-species sequence, to which diffusion of $X = HBrO_2$ and $Y = Br^-$ is added, with the reasonable simplifying assumption of equal diffusion coefficients. Thus the kinetic equations (B.11)–(B.13) become

$$\frac{\partial u}{\partial t} = k_1 a v - k_2 u v + k_3 a u - k_4 u^2 + D\frac{\partial^2 u}{\partial r^2} \tag{B.27}$$

$$\frac{\partial v}{\partial t} = -k_1 a v - k_2 u v + f k_5 w + D\frac{\partial^2 v}{\partial r^2} \tag{B.28}$$

with D the diffusion coefficient of the two species, r the (radial) spatial variable, treating this as a one-dimensional problem, and the other variables defined as before. It is useful, as usual, to nondimensionalize the system; a further fruitful simplication occurs if we then note that the nondimensionalized terms corresponding to the $\pm k_1 a v$ terms, the first on the right-hand-sides of (B.27) and (B.28), are small and may be neglected. Then we arrive at a set of nondimensionalized model (reaction-diffusion) equations for the leading edge of the travelling waves in the BZ reaction, namely

$$\frac{\partial x}{\partial t} = x(1 - x - sy) + \frac{\partial^2 x}{\partial r^2}, \tag{B.29}$$

$$\frac{\partial y}{\partial t} = -bxy + \frac{\partial^2 y}{\partial r^2}, \tag{B.30}$$

where s and b are positive parameters, of $O(1)$ (a numerical study of this system has been carried out by Manoranjan and Mitchell [219]).

We proceed by looking for *travelling wavefront solutions* of these equations, where the bromous acid concentration $[HBrO_2]$ moves from a region of high value to low value as it reduces the level of the bromide ion. Thus we seek solutions of the form $x(r,t) = f(\xi)$, $y(r,t) = g(\xi)$, where $\xi = r + ct$ for some wave speed c, and with the appropriate boundary conditions. Substituting this form of the solutions into the above equations, we obtain a system of ordinary differential equations in ξ, which is still fairly intractable analytically, but which enables bounds and estimates on the wave speed and width of the wavefront to be obtained, which agree fairly well with the experimentally observed values.

In particular, values for the wave speed of the order of 2.7 mm min^{-1} are obtained, which are standard values for reaction-diffusion waves. Note that this gives a time of about 4 minutes for a wave to travel 1 cm, as opposed to a diffusional time of $O(1cm^2/D)$, or about 850 minutes for a

diffusion constant of $D \approx 2\times10^{-5}$ cm^{2}s^{-1}, typical for small molecules such as the ones considered here. Thus, as a means of transmitting information via a change in chemical concentration, reaction-diffusion waves are orders of magnitude faster than pure diffusion, if the distances involved are other than very small; on the other hand, for distances of the order of cell diameters which may be typical in embryology, diffusion may be relevant [251, pp.327–328].

Spiral Waves

A different form of wave pattern produced in the BZ reaction is the spiral wave: a rotating, time-periodic, spatial structure of reactant concentrations, such that a snapshot at any fixed time shows a spiral pattern. A movie of the entire process shows the whole spiral pattern moving like a rotating clock spring. The spiral patterns may be symmetric, in a clockwise or anticlockwise direction, or considerably more complex; multi-armed spirals are also observed. The symmetry-breaking associated with spiral waves is of a different form from that of target patterns — here we have the introduction of a fixed *chirality*, or direction of rotation, leading to an intrinsic asymmetry such as is observed in the natural chirality observed in much of biology, especially in the handedness of fundamental biochemical molecules.

Analysis of Spiral Waves A spiral wave may be treated mathematically by considering that, at any fixed position in the medium, it seems locally as if a periodic wave train is passing by since every time the spiral turns a wave front moves past. Also, it is clearly appropriate to use polar coordinates, r and θ, when discussing spiral waves. Thus a simple rotating spiral may be described by a periodic function of the phase ϕ where

$$\phi = \Omega t \pm m\theta + \psi(r), \tag{B.31}$$

where Ω is the frequency, m is the number of arms on the spiral, $\psi(r)$ is a function which describes the kind of spiral, and the $\pm$ in the $m\theta$ term determines the sense of rotation.

Spiral wave solutions of the reaction-diffusion equations describing the BZ reaction may be sought by looking for a solution of the above form; but the analysis is very complex. For a simple class of reaction-diffusion systems, called λ–ω systems, a general analysis of the existence of spiral waves has been performed; an early analysis was by Cohen, Neu and Rosales [62]. An important and difficult problem was to account for the fact that spiral waves have unique frequency and curvature, so that they must satisfy both the dispersion relation (giving the wave speed as a function of the period or frequency), and a geometrical constraint on the curvature of propagating wavefronts in two-dimensional media; this is treated by Keener and Tyson [183]. The analysis of spiral waves in general requires much use of asymptotic techniques; there is by now an extensive literature on such spiral wave solutions in reaction-diffusion systems (see the above references and [251], and sources quoted therein).

Spiral Wave Simulations It is of interest to note that spiral patterns analogous to those observed in the BZ reaction have been obtained in computer simulations using discrete models, or cellular automata, together with some simple initial conditions and rules for the dynamics of cells based on the states of their neighbours (that is contact-mediated communication rather than diffusion is used for information transfer) — see for example [79, 109, 213]. The early cellular

automaton models were based merely on heuristically derived rules that were able to simulate the patterns, with no underlying correspondence with the experimental mechanisms, but more recent models such as in [109] are based on excitable media and feasible local interactions. For a further discussion of discrete models and cellular automata, see section 6.2; the exact nature of their correspondence to continuous partial differential equation models (such as reaction-diffusion models) is not yet quite clear.

More Complex Structures As might be anticipated, in three dimensions the BZ reaction displays an even wider variety of interesting structures; unfortunately, furthermore as expected, the analytical study of these is even more intricate than in lower-dimensional cases. Isoconcentration surfaces including helical waves, toroidal and twisted toroidal scroll waves, and more intricate spherical-like structures have been observed. For photographs of actual three-dimensional waves, see [362]; for an analysis of the wave solutions in three dimensions, see for example [123], and for a discussion of the topological aspects, refer to [381].

For the case of purely temporal oscillations, it was noted above that complex and chaotic behaviour might result under certain conditions. In the case of chemical waves, this is, unsurprisingly, also the case, extending the temporal results to the spatio-temporal domain. This behaviour is manifested in the response to an electric field: The spatially nonhomogeneous concentration profiles involved in the wave patterns discussed above give rise to electric potential gradients as ionic species participate in the spatial patterning. It is thus of interest to study the interaction, for example, of the reaction-diffusion system with an imposed electric field, so that our kinetic equations must consider mass transport both due to molecular diffusion and due to ionic migration in the applied field. A periodically applied potential has been shown to induce simple, complex and chaotic wave trains, the last being characterised by chaotic features such as a 'devil's staircase'-like dependence of the pulse firing number on the amplitude or frequency of the perturbation — see for example [220] and references therein. Once again, we observe the interplay and deep connection between the spontaneous order in self-organizing systems such as reaction-diffusion systems, and the 'disorder' and absence of predictability associated with chaotic behaviour.

Further evidence for dissipative and self-organized structure is demonstrated by observed hysteresis effects: The FN model (B.11)-(B.13) for the BZ reaction displays only one nontrivial steady state, but this appears to be a simplification, and multiple steady states have been reported, which are seemingly lost in the approximations that go into the FKN mechanism and the FN model; see [337] and references therein. As a result, experiments performed in coupled cells may have structures formed with one steady state in some regions and another elsewhere, with appropriate linking conditions between the steady states. The consequently observed phenomena of multiple steady states, a 'choice' which depends on initial conditions, hysteresis and 'memory', are again characteristics of self-organization.

Thus the BZ reaction displays a variety of spatio-temporal symmetry-breaking phenomena, which accord well with the approximate analytical results obtained; for this reason it is a popular paradigm for chemical oscillations and pattern formation, and for self-organizing, or dissipative structure in general. What, then, of purely spatial dissipative structures, of stationary spatial pattern formation?

B.3.3 Stationary Concentration Patterns

In the context of the formation of 'dissipative structures' and self-organization, one of the most important results is the possibility of the spontaneous appearance of stable spatially inhomogeneous, but stationary, structures in an initially homogeneous system. Such behaviour has been shown to be possible in reaction-diffusion systems (see appendix A), which encompass the Oregonator equations which so successfully describe the spatio-temporal structures arising in the BZ reaction. Thus the search has naturally been on to demonstrate the existence of stable spatial patterns in chemical systems, a quest which has only recently borne fruit.

There is extensive mathematical support for the feasibility of formation of nonequilibrium stationary concentration patterns, or Turing structures [354] — see appendix A and section 3.2. The mechanism of formation of these structures is based on the simple but counterintuitive notion that diffusion does not necessarily increase the spatial uniformity of chemical reactions. If some reactants diffuse faster than others and if the faster species inhibit reaction while the slower ones are autocatalytic, spatial patterns may spontaneously emerge by a symmetry-breaking instability of the uniform steady state. By this means, spontaneous emergence of patterns is possible, a mechanism which Turing proposed could explain the development of spots and stripes, and other biological features, in embryonic development, an aspect which is a major concern of chapter 4 of this thesis. There has been a great deal of theoretical interest in developmental biology in this proposed mechanism for pattern formation — for examples, see chapter 4 — but the acceptance of this mechanism was long hampered by the lack of experimental proof that such stationary pattern formation was possible in a chemical reaction at all.

Stationary Patterns in the BZ Reaction? The BZ chemical 'rotor' provides clear evidence for reaction-diffusion pattern formation, as discussed fairly extensively above, for instance in the dramatic, spiral, propagating wave that may be emitted by the localized region of chemical excitation. The mechanism for this is well-understood, however, and does not involve the Turing bifurcation — the BZ rotor moves, does not arise spontaneously from the antecedent uniform steady state, and does not require unequal diffusion [368]. There were some experimental indications for stationary spatial pattern formation in the BZ reaction, in a ferroin-catalyzed system [325], and in an uncatalyzed reagent [284], but interpretation of the experiments was clouded by convective and surface effects, so that it was not clear that the patterns were maintained only by the interaction of reaction and diffusion, rather than by hydrodynamic convection.

A theoretical analysis [24] provided support for the formation of stationary patterns in the Oregonator model of the BZ reaction; the small-amplitude patterns, calculated by a perturbation technique, may be long-lived but are ultimately unstable, whereas the large-amplitude patterns (in one dimension) were calculated numerically and considered to be stable. However, these calculations demonstrated structure formation only if the diffusivities differ by a factor of at least ten, which is not known to be the case in the BZ reaction, where the molecules all have more or less the same diffusion coefficients. No clear-cut Turing structures (stable, maintained only through the interaction of reaction and diffusion, and arising spontaneously from the homogeneous steady state) have been demonstrated for the BZ reaction, nor may these be expected, due to the requirement of varying diffusion coefficients — computations on the BZ reaction predict pattern formation only when the relative values of the diffusion coefficients are in contradiction with physical arguments.

Stationary Turing Structures in the CIMA Reaction

In the search for Turing-type structures in chemical systems, this requirement of varying diffusivities proved difficult to satisfy, as in aqueous solution all molecules diffuse at more or less the same rate; thus there developed a growing scepticism about the physical reality of such structures [308]. Recently, however, various groups have managed to design an experimental arrangement in which this critical condition was satisfied. The reaction involved is the chlorite-iodide-malonic acid, or CIMA reaction, devised as a substitute for the BZ oscillator. The experimental setup involved diffusion along a gel; as chemicals reacted with one another, the concentrations of the reagents could be observed from the colour of the gel, which was concentration-dependent, varying from yellow to blue.

More specifically, a strip of gel loaded with starch indicator was fed with constant concentrations of malonic acid (MA) at one end and chlorite (ClO_2^-) and iodide (I^-) ions at the other end. As the reactants diffuse towards each other, patterns which are nonuniform both parallel and perpendicular to the direction along which the reactants diffuse toward each other were observed in the colour of the starch-I_3^- complex [51]. The patterns satisfy the requirements for a Turing structure — they result from *spontaneous symmetry-breaking* of steady states, they are *stationary, stable* structures arising from the sole *coupling of reaction and diffusion* processes, and are characterized by an *intrinsic wavelength* independent of the geometrical parameters (except for small domain sizes, smaller than a few wavelengths, where the necessary fit with the boundary conditions influences the pattern wavelength — see appendix A.4) — and hence provide the first fairly incontrovertible evidence of Turing symmetry-breaking structures [51].

The necessary step to demonstrate that Turing bifurcation was involved was to model the reaction, and show that the necessary conditions were satisfied [308] — and indeed, the mechanism of the CIMA reaction in the above experimental setup was shown to be modelled by a reaction-diffusion system. Furthermore, the key to the success of the experiment [51] was in how the reacting chemicals diffuse through the gel: The inhibitor molecules, in this case ClO_2^- ions, do not interact with the gel, but instead move unimpeded through the liquid that permeates the gel; the activator molecules (iodine ions) on the other hand, interact chemically with the gel, forming short-lived or long-lived complexes, and this slows them down. Thus the requirement of greatly varying diffusivities is satisfied, and a Turing bifurcation and associated pattern formation may occur — "...we finally have a nice clean example of Turing structures in chemistry with a nice, clean theory to go along with it" (Tyson, quoted in [308]).

The feature of the CIMA reaction, discussed above, which allows the pattern formation, may be somewhat rare in inorganic reactions, in that the 'inert medium', the gel, makes it possible to establish the crucial difference in the effective diffusion coefficients of activator and inhibitor. On the other hand, in biological systems, membrane-bound species play key roles, so that binding and associated ranges in diffusivities are far more likely; furthermore, substrate inhibition, rather than autocatalysis, is a common means of dynamical regulation. In this case, Turing bifurcation indeed appears likely to occur and to be a major mechanism for pattern formation — for a more thorough discussion, see chapter 4.

Additional Experimental Support — the Bifurcation Point Further work on the CIMA reaction and associated systems in recent years has supported and strengthened the conclusions

that Turing bifurcations and spatial self-organization are involved. One of the features of the Turing bifurcation demonstrated in the mathematical analyses (appendix A) is the existence of a bifurcation point, depending on diffusion coefficients and reaction rates, such that spontaneous pattern formation can occur, say, below it and not above it.

The existence of such a point has been demonstrated experimentally: Reaction rates are temperature dependent, each with its own temperature dependence (while diffusion coefficients are less temperature-sensitive than typical reaction rates), so Ouyang and Swinney [299] used temperature to tune the CIMA reaction, assuming that the Turing bifurcation point depends on a control parameter, in this case temperature. When temperature was taken below the critical 18°C, hexagonal, striped or mixed patterns with an intrinsic wavelength were observed, which were lost again if the temperature was raised again above 18°C. No hysteresis was obtained (some might be expected in the absence of specific symmetries, but numerical simulations indicate that the hysteresis is mostly too small to be observed in the experimental setup); the pattern emerges slowly near the threshold, and the amplitude increases with increasing distance of the control parameter from the threshold — all as theory requires. Two-dimensional chemical patterns similar to those observed [299] have been found in numerical simulations of reaction-diffusion systems, such as the Brusselator or activator-inhibitor models. Thus patterns, similar to those predicted by theory, have been demonstrated to emerge by a spontaneous symmetry-breaking instability from initial uniformity, with a vigour proportional to the excess of some parameter over a threshold, lending strong experimental support to the existence of Turing bifurcation [368].

Transient Turing Structures in Closed Systems The above experiments indicating stable spatial patterns were all performed, for practical reasons, in open systems with initial gradients of the reactants being fed into the reactor (strip of gel). Transient, symmetry-breaking spatial patterns have recently been obtained in a closed, gradient-free, aqueous medium, also using the CIMA reaction [201]. This appears even closer to the (mathematical) analyses of Turing structures, which envision systems with no imposed gradients, in which the key reactants are maintained at uniform concentrations throughout the medium. Again the mechanistic analysis and associated mathematical study of the system supports the creation of Turing structures. Because the system is closed, the structures are necessarily transient — they require about 25 minutes to form, and remain stationary for 10 to 30 minutes, before decaying [201].

This scenario seems to provide even more support for the search for Turing structures in development, as Turing bifurcations in developing embryos are more likely to be in closed systems, arising transiently from nearly uniform concentrations, than in a situation in which different reactants enter from opposite ends of the relevant parts of the embryo. The fact that the structures are transient should not be too much of a conceptual problem, as the patterns can be 'frozen in' by the developing system, through developmentally determined changes in its chemical environment; significant is the fact that such transient Turing structures have been shown to form at all.

Thus there has, in the last three years, emerged strong experimental support for the existence of stable and transient Turing structures in chemical systems, arising from symmetry-breaking instabilities and entailing spatial self-organization; they are adequately modelled by reaction-diffusion systems. These results, in tandem with the results on spatio-temporal self-organization

obtained in the BZ reaction, strongly support the existence of dissipative structures (to use Prigogine's terminology), and provide a paradigm for self-organization. As the conditions under which such pattern formation occurs appear, at least on the face of it, to be satisfied in developing embryos, the chemical demonstrations of self-organization seem to provide strong circumstantial evidence for the concept of self-organized pattern formation in development by Turing instabilities or analogous mechanisms. The detailed application of these concepts to specific developmental situations is dealt with in chapter 4.

Appendix C

Mechanochemical Models for Pattern Formation

As indicated in chapter 5, there is a range of models for spontaneous pattern formation that is based on the properties of mesenchymal or epithelial cellular behaviour, in which the cells play an integral role in the formation of structure. The mathematical formulation of such models is frequently more complex than that of reaction-diffusion equations, and thus displays a wider range of solution behaviour that, however, still falls within the symmetry-breaking, self-organization paradigm.

C.1 Mechanical Model for Mesenchymal Morphogenesis

The first such model to be given a detailed mathematical formulation and analysis was the mechanical model for mesenchymal morphogenesis, discussed in section 5.1.1, which was proposed by Oster, Murray and Harris in 1983 [259, 289]. We will proceed to give a derivation of the model equations (5.1)–(5.3), which are simply stated in the text. For these derivations and discussions, we closely follow the various presentations by Murray and coworkers (for example [251, 255, 257, 289]).

C.1.1 Derivation of Model Equations

The basic mathematical model for mesenchymal morphogenesis consists of three equations, governing (i) the conservation equation for cell population density; (ii) the mechanical balance of forces between the cells and the extracellular matrix (ECM); and (iii) the conservation law governing the ECM. We will treat each of these separately, taking into account the various interactions and factors which can contribute to cell motion and matrix deformation, as discussed in the text (see sections 2.1.2, 2.1.3 and 5.1.1).

Thus, let $n(\mathbf{r}, t)$ and $\rho(\mathbf{r}, t)$ denote respectively the cell and ECM density at position $\mathbf{r}$ and time t; and let $\mathbf{u}(\mathbf{r}, t)$ be the displacement vector of the ECM — that is, a material point in the matrix initially at position $\mathbf{r}$ undergoes a displacement to $\mathbf{r} + \mathbf{u}$.

Cell Conservation Equation

The general form of the conservation equation is

$$\frac{\partial n}{\partial t} = -\nabla \cdot \mathbf{J} + M, \tag{C.1}$$

where $\mathbf{J}$ is the flux of cells (number crossing unit area in unit time) and M is the mitotic or cell proliferation rate.

The specific form of the mitotic term M is not important [257]; for simplicity a logistic growth model, $M = rn(N - n)$, may be chosen, where r is the initial cell proliferation rate, for small n, and N represents a maximum cell density or carrying capacity in the absence of other effects. The mitotic rate has been shown to be influenced by cell shape; to include this effect the model might have to be extended to include a dependence of M on $\mathbf{u}$.

A number of factors which affect cell motion are included in $\mathbf{J}$:

Convection: The passive movement of cells being dragged along by the matrix (which itself is deformed by cell tractions) gives the convective flux $\mathbf{J}_c$, which is simply the product of the cell density and the local matrix velocity:

$$\mathbf{J}_c = n\frac{\partial \mathbf{u}}{\partial t}. \tag{C.2}$$

Random Dispersal: In a homogeneous medium cells tend to disperse randomly; this is generally modelled in the manner of classical diffusion using Fick's law (see section 4.2.1), giving a flux term $-D_1 \nabla n$ ($D_1 > 0$). This corresponds to random motion in which the cells respond to local cell density variations. This formulation is not by itself sufficient, however; the high densities in the developing embryos, and the long-range interactions possible through the long filopodia which extend beyond nearest neighbours, mean that *nonlocal* effects on diffusive dispersal should be included as well. It may be shown (see the discussion in [251]) that the appropriate expression for the diffusive flux of cells which respond to local concentration averages is given by

$$\mathbf{J}_D = -D_1 \nabla n + D_2 \nabla(\nabla^2 n). \tag{C.3}$$

Here the second term gives rise to a *biharmonic* term in the conservation equation, which is stabilizing if the long-range diffusion coefficient $D_2 > 0$.

The tendency of cell motion to follow geometrical cues in their substratum, for instance moving along aligned fibres or grooves, has been well-established through numerous experiments; this property is denoted by *contact guidance.* In the case of the interaction of cells and ECM, the cells will tend to follow the alignment of the matrix (due to adhesive interactions — see section 2.1.2). This may be most simply modelled by a biased random walk, in which the diffusion coefficients D_1 and D_2 are functions of the ECM strain ε, which is a measure of the matrix deformation. This somewhat complicated effect may however be neglected, to first order, if we are only concerned with a linear stability analysis.

Haptotaxis: The traction exerted by the cells on the matrix generates gradients in the matrix density, $\rho(\mathbf{r}, t)$, and hence in the density of adhesive sites for cell attachment. The net displacement of cells which extend filopodia randomly is in the direction in which they can get the strongest grip; that is, *up* a matrix density gradient. Because of the nonlocal sensing properties

of the cells, a long-range effect similar to the biharmonic diffusion term should probably be included. Thus the haptotactic flux becomes

$$\mathbf{J}_h = n\nabla(a_1\rho - a_2\nabla^2\rho), \tag{C.4}$$

where $a_1 > 0$, $a_2 > 0$.

As noted in the text, a number of plausible contributions to the flux, including galvanotaxis (motion in response to an electric potential gradient) and chemotaxis (due to the gradient in some attracting or repelling chemical — see appendix C.3), have been omitted; possible forms for these terms are given by Murray [251].

With the above contributions to the cell motion and the illustrative logistic term for mitosis, the cell conservation equation becomes

$$\begin{aligned}\frac{\partial n}{\partial t} &= \underbrace{-\nabla\cdot\left[n\frac{\partial \mathbf{u}}{\partial t}\right]}_{\text{convection}} + \underbrace{\nabla\cdot[D_1\nabla n - D_2\nabla(\nabla^2 n)]}_{\text{diffusion}} \\ &\quad \underbrace{-\nabla\cdot[a_1\nabla\rho - a_2\nabla(\nabla^2\rho)]}_{\text{haptotaxis}} + \underbrace{rn(N-n)}_{\text{mitosis}},\end{aligned} \tag{C.5}$$

where D_1, D_2, a_1, a_2, r and N are positive parameters.

Cell-Matrix Mechanical Interaction Equation

The structure and composition of the fibrous extracellular matrix, or ECM, are complex, and change as development proceeds (see section 2.1.2). It is thus not feasible to attempt to model the mechanical interaction between the cells and matrix in detail. As the deformations are small, however, it is reasonable as a *first approximation* to model the composite material of cells plus matrix as a linear, isotropic viscoelastic continuum, with stress tensor $\sigma(\mathbf{r}, t)$.

As the time scale of embryonic motions during development is long, of the order of hours, and the spatial scale is very small, less than a few millimetres, we are in a small Reynolds number regime (see for example [283]), and can ignore inertial effects in the cell-ECM interaction equation. Thus we take the tractional forces generated by the cells to be in mechanical equilibrium with the viscoelastic restoring forces developed in the matrix, and with any external forces present. Then the mechanical cell-matrix equation is

$$\nabla\cdot\sigma + \rho\mathbf{F} = \mathbf{0}, \tag{C.6}$$

where $\mathbf{F}$ is the external force (per unit matrix density) acting on the matrix.

The *body forces* contained in $\mathbf{F}$ include the restraining forces responding to the displacement of the matrix from its equilibrium position, as the matrix is attached to some substratum of underlying tissue, such as epidermis. To first approximation, these forces are proportional to the displacement, that is

$$\mathbf{F} = -s\mathbf{u}, \tag{C.7}$$

where $s > 0$ is an elastic parameter characterizing the substrate attachments.

There are two contributions to the stress tensor σ, due to the *deformations* of the matrix, modelled as a linear viscoelastic material, and due to *cell traction*:

$$\sigma = \sigma_{\text{ECM}} + \sigma_{\text{cell}}. \tag{C.8}$$

Matrix Deformations: For the first term in the stress tensor, we use the usual expression for a linear viscoelastic material, giving the stress-strain constitutive relation as

$$\sigma_{\text{ECM}} = \underbrace{[\mu_1 \varepsilon_t + \mu_2 \theta_t \mathbf{I}]}_{\text{viscous}} + \underbrace{E'[\varepsilon + \nu' \theta \mathbf{I}]}_{\text{elastic}}, \tag{C.9}$$

where

$$E' = E/(1+\nu), \quad \nu' = \nu/(1-2\nu), \tag{C.10}$$

and E and ν are Young's modulus and Poisson's ratio, respectively, $\mathbf{I}$ is the unit tensor, and μ_1 and μ_2 are the shear and bulk viscosities of the ECM. Also, ε and θ are respectively the strain tensor and dilation of the matrix, defined by

$$\varepsilon = \tfrac{1}{2}(\nabla \mathbf{u} + \nabla \mathbf{u}^T), \quad \theta = \nabla \cdot \mathbf{u} \tag{C.11}$$

(superscript T denotes the matrix transpose, and subscript t a partial time derivative).

The above formulation is certainly a simplification; fibrous materials such as those we are considering are also characterized by *nonlocal* elastic interactions, since the fibres can transmit stress between points in the ECM quite far apart; thus we could include long-range effects into σ_{ECM} by the incorporation of biharmonic terms analogous to those in the cell equation. We could also introduce *anisotropy* by having a θ-dependence for E and ν, in particular, letting $E(\theta)$ be an increasing function of θ. For the simplest model, however, it suffices to ignore these refinements.

Tractional Stresses: The contribution to the stress tensor from the cell tractive force, σ_{cell}, must initially increase with n — the more cells there are, the greater the traction. Experimental evidence for *contact inhibition*, however, indicates that the traction force decreases for large enough cell densities. Such behaviour can most simply be modelled by letting the measure of the traction force generated by a cell, $\tau(n)$, be given by

$$\tau(n) = \frac{\tau n}{1 + \lambda n^2}, \tag{C.12}$$

where $\lambda > 0$, and $\tau > 0$ is a traction parameter which will be crucial to our subsequent analysis. As the filopodia with which the cells attach to the ECM probably extend beyond the immediate neighbourhood of the cells, it is again reasonable to include a nonlocal effect. Thus the stress contribution due to traction (which is assumed to be isotropic) may be taken to be

$$\sigma_{\text{cell}} = \frac{\tau n}{1 + \lambda n^2}(\rho + \gamma \nabla^2 \rho)\mathbf{I}, \tag{C.13}$$

where $\gamma > 0$ is a measure of the nonlocal long-range cell-ECM interactions; these turn out to be fairly significant in the model behaviour.

Thus the cell-matrix force equation (C.8) becomes, with the inclusion of the above terms,

$$\nabla \cdot \underbrace{[\mu_1 \varepsilon_t + \mu_2 \theta_t \mathbf{I}]}_{\text{viscous}} + \underbrace{E'(\varepsilon + \nu' \theta \mathbf{I})}_{\text{elastic}} + \underbrace{\tau n (1 + \lambda n^2)^{-1}(\rho + \gamma \nabla^2 \rho)\mathbf{I}]}_{\text{cell traction}} - \underbrace{s\rho \mathbf{u}}_{\text{external forces}} = \mathbf{0}, \tag{C.14}$$

with the parameters defined as above.

Matrix Conservation Equation

The conservation equation for the matrix density $\rho(\mathbf{r}, t)$ is simply given by

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}_t) = S(n, \rho, \mathbf{u}). \tag{C.15}$$

This equation embodies the assumption that the matrix moves only by convection. The term $S(n, \rho, \mathbf{u})$ is the rate of secretion of matrix by cells, which plays a role in some applications, particularly in wound healing, but is generally negligible on the time scale of cell motions that we shall be considering; so we can henceforth assume that $S = 0$.

Equations (C.5), (C.14) and (C.15) constitute the 'field equations' for the model pattern formation mechanism for mesenchymal cells, involving a range of parameters, all of which are in principle experimentally measurable. As was done above for the reaction-diffusion models analysed, we *nondimensionalize* the equations to assess the relative importance of the various effects. Thus, following [257], we introduce a typical length scale L and time scale T, and a typical matrix density ρ_0, and define the following dimensionless variables and parameters:

$$\begin{aligned}
&\mathbf{r}^* = \mathbf{r}/L, \quad t^* = t/T, \quad n^* = n/N, \quad \mathbf{u}^* = \mathbf{u}/L, \quad \rho^* = \rho/\rho_0, \\
&\nabla^* = L\nabla, \quad \theta^* = \theta, \quad \varepsilon^* = \varepsilon, \quad \gamma^* = \gamma/L^2, \quad r^* = rNT, \\
&s^* = s\rho_0 L^2(1+\nu)/E, \quad \lambda^* = \lambda N^2, \quad tau^* = \tau\rho_0 N(1+\nu)/E, \\
&a_i^* = a_i\rho_0 T/L^2, \quad \mu_i^* = \mu_i(1+\nu)/TE, \quad (i = 1, 2), \\
&D_1^* = D_1 T/L^2, \quad D_2^* = D_2 T/L^4.
\end{aligned} \tag{C.16}$$

With these definitions, and after dropping the asterisks for notational simplicity, the dimensionless equations become

$$\begin{aligned}
n_t = {} & D_1\nabla^2 n - D_2\nabla^4 n - \nabla \cdot [a_1 n\nabla\rho - a_2 n\nabla(\nabla^2\rho)] \\
& -\nabla \cdot (n\mathbf{u}_t) + rn(1-n),
\end{aligned} \tag{C.17}$$

$$\nabla \cdot \left\{ (\mu_1\varepsilon_t + \mu_2\theta_t \mathbf{I}) + (\varepsilon + \nu'\theta\mathbf{I}) + \frac{\tau n}{1+\lambda n^2}(\rho + \gamma\nabla^2\rho)\mathbf{I} \right\} = s\rho\mathbf{u}, \tag{C.18}$$

$$\rho_t + \nabla \cdot (\rho\mathbf{u}_t) = 0. \tag{C.19}$$

The number of dimensionless parameters can be reduced further by choosing appropriate length and time scales; for example, if we wish to study the system evolution on the order of mitotic times, we could set $r^* \equiv 1$, that is, $T = 1/rN$. All dimensionless parameters are positive, and they may be divided into those associated with the *cell properties*, namely a_1, a_2, D_1, D_2, r, τ, and λ, and those related to the *matrix properties*, namely μ_1, μ_2, ν', γ and s.

The model does not include all relevant effects, as has already been pointed out; but is intended to illustrate the type of model that may be constructed for the consideration of mesenchymal morphogenesis. The terms which are included or retained in any particular application will depend on the specific case.

C.1.2 Analysis and Pattern Formation Potential of Mechanical Model

We have presented the derivation of the various terms in the above equation to illustrate the formulation of such a more complex model, in which the cells participate intimately in structure

formation. It is clear that many variants of such models are possible, in which different factors are given more or less attention in the model formulation. The further analysis of an equation system such as (C.17)–(C.19) proceeds by techniques with which we are already familiar; for instance, linear stability analysis is used to determine any potential for departure from spatial homogeneity; and then nonlinear bifurcation or perturbation analyses are applied in an attempt to gain an insight on the form of the nonlinear solutions. Such analytic techniques may obviously be complemented by numerical simulation of the equations. A specific goal for such studies is to determine the pattern-forming potential displayed by individual terms in the model, such as the effects of varying, say, cell traction or mitosis.

The methods used are thus analogous to those we have already encountered in connection with reaction-diffusion equations. The significant difference, however, is the greatly heightened complexity of these equations, with an associated analytical intractability; there is little hope at this stage of finding useful analytical solutions to the full system (C.17)–(C.19). In order to gain some insight, we shall see that it has proved to be useful to neglect some of the terms in the equations to obtain simpler models, which can provide glimpses of the potentialities of the full system.

Linear Stability Analysis and Dispersion Relation

The nondimensional model equation system (C.17)–(C.19) has uniform steady state solutions given by $\mathbf{u} = \mathbf{0}$, $\rho =$ constant, and $n = 0$ or $n = 1$. Clearly, $\rho = 0$ or $n = 0$ are not relevant to the biological situation, as the matrix and cell densities must be finite; thus the physically reasonable steady state is $\mathbf{u} = \mathbf{0}$, $n = \rho = 1$ (where $\rho = 1$ follows from a suitable choice of the uniform initial matrix density ρ_0 in the nondimensionalization). The linear stability analysis then proceeds in the usual way, by considering small deviations $\tilde{n}$, $\tilde{\rho}$ and $\tilde{\mathbf{u}}$ from the steady state values, and retaining only linear terms (dropping the tilde for notational convenience):

$$n_t - D_1\nabla^2 n + D_2\nabla^4 n + a_1\nabla^2\rho - a_2\nabla^4\rho + \theta_t + rn = 0, \tag{C.20}$$

$$\nabla\cdot[(\mu_1\varepsilon_t + \mu_2\theta_t\mathbf{I} + (\varepsilon + \nu'\theta\mathbf{I}) + (\tau_1 n + \tau_2\rho + \tau_1\gamma\nabla^2\rho)\mathbf{I}] - s\mathbf{u} = 0, \tag{C.21}$$

$$\rho_t + \theta_t = 0, \tag{C.22}$$

where

$$\tau_1 = \frac{\tau}{1+\lambda}, \quad \tau_2 = \frac{\tau(1-\lambda)}{(1+\lambda)^2}. \tag{C.23}$$

To determine the stability criteria, we look as usual for solutions of the form n, $\mathbf{u}$, and ρ proportional to $\exp(\sigma t + i\mathbf{k}\cdot\mathbf{x})$; then substitution into (C.20)–(C.22) gives the *dispersion relation* determining σ as a function of the wave vector $\mathbf{k}$:

$$\sigma[\mu k^2\sigma^2 + b(k^2)\sigma + c(k^2)] = 0, \tag{C.24}$$

where

$$b(k^2) = \mu D_2 k^6 + (\mu D_1 + \gamma\tau_1)k^4 + (1 + \mu r - \tau_1 - \tau_2)k^2 + s, \tag{C.25}$$

$$\begin{aligned} c(k^2) = {} & \gamma\tau_1 D_2 k^8 + (\gamma\tau_1 D_1 - \tau_2 D_2 + D_2 - a_2\tau_1)k^6 \\ & + (D_1 + sD_2 - \tau_2 D_1 + \gamma\tau_1 r - a_1\tau_1)k^4 \\ & + (r + sD_1 - r\tau_2)k^2 + rs. \end{aligned} \tag{C.26}$$

Here we have set $\mu = \mu_1 + \mu_2$; τ_1, τ_2, μ and s replace $\tau_1/(1+\nu')$, $\tau_2/(1+\nu')$, $\mu/(1+\nu')$ and $s/(1+\nu')$, respectively, for notational convenience; and $k = |\mathrm{k}|$.

The Dispersion Relation As we have seen in appendix A.4, the dispersion relation gives information about the stability of the uniform solution, and about the wave numbers of the perturbed, unstable solutions. We see from the dispersion relation (C.24) that $\sigma < 0$ for $k^2 = 0$, so that the uniform solution is linearly stable. Spatially heterogeneous solutions of the linear system are characterized by a dispersion relation $\sigma(k^2)$ which exhibits a range of unstable modes for which Re $\sigma(\mathrm{k}^2) > 0$, for $k^2 \neq 0$. For values of k for which this instability relation holds, finite amplitude spatially structured solutions are expected to evolve from the uniform steady state in the face of small random perturbations, giving rise to symmetry-breaking and self-organization as in the case of reaction-diffusion systems; the solutions will not grow in an unbounded manner due to contact inhibition and logistic damping.

The analysis of the dispersion relation proceeds as for reaction-diffusion equations: as before, conditions for pattern formation are $b(k^2) < 0$ or $c(k^2) < 0$, since then Re $(\sigma) > 0$ for such k. This gives ranges of the parameters (analogous to the Turing space) — or more correctly, ranges for the dimensionless groupings or combinations of the parameters — for which pattern formation is possible. The intricacy of the above mechanical equations indicates, however, that considerably more complicated parameter dependencies and solution behaviour may be obtained here than for the reaction-diffusion systems, so that these equations constitute largely analytically difficult or intractable and hence unexplored terrain.

Overview of Analytic Approaches and Results Some analyses have however been performed — for detailed overviews of the results that have been obtained, see the original papers or existing reviews, for example [251, 255, 257] — indicating some of the pattern-forming potential of this model for mesenchymal morphogenesis. Owing to the complexity of the system, in general the solution behaviour and effect of various parameters is best elucidated for special cases, in which some parameters are considered in isolation, with others set equal to zero. Such analyses permit one to see, for example, which terms dominate or are essentially redundant for pattern formation. Under such restricted conditions, a wide range of dispersion relations has been derived [257], suggesting a considerable diversity of k-dependencies and hence patterning possibilities, much greater than for reaction-diffusion systems. In particular, there are dispersion relations $\sigma(k^2)$ which suggest pattern formation for k approaching zero or infinite ranges of k values, so that patterning can occur, for instance, for arbitrarily small or large k^2. Some dispersion relations also exhibit singularities, in which the growth rate predicted by linear theory becomes infinite; simplified, caricature models with similar singular dispersion relations have been studied as singular perturbation problems using bifurcation and asymptotic analyses [41].

The qualitative morphogenetic effects of the various parameters are not easily isolated and characterized, but some conclusions may be drawn from looking at the expressions for $b(k^2)$ and $c(k^2)$, since we require one of these two to become negative for pattern formation. We can see, for example, that the tethering to some substratum, quantified by s, tends to stabilize the homogeneous solution, since it is a positive term in both b and c; similarly, the viscosity is stabilizing, while long-range haptotaxis, contained in a_2, is destabilizing. Of particular importance is the fact that the only negative terms in the expressions for b and c are those with τ_1

and τ_2, both involving the traction parameter τ. That is, the cell traction forces are the only contribution to the aggregative process in the force balance equation (C.18). Hence, according to the model, traction plays a central, crucial role in the patterning process, in agreement with the experimental *in vitro* observations of the morphogenetic properties of traction, as reported by Harris and coworkers [147, 148] (see section 5.1.1).

Analysis of a Simplified Model

As an illustrative example, in the context of our overall study of self-organization, it is useful to consider briefly a simplified model which presents the possibility of spatial structuring, to demonstrate bifurcation from the spatially uniform equilibrium to spatial heterogeneity; extrapolation to the full system, as indicated, clearly admits a much wider range of pattern-forming possibilities. Thus consider the case where cell diffusion and haptotaxis, as well as cell division, are neglected; the implication is that cell convection by the matrix constitutes the major transport process. In the system equations, set $D_1 = D_2 = a_1 = a_2 = r = 0$, to derive the simplified equations. Then the system linearizes (in one space dimension) to

$$\begin{aligned} &n_t + u_{tx} = 0, \\ &\mu u_{xxt} + u_{xx} + [\tau_1 n + \tau_2 \rho + \tau_1 \gamma \rho_{xx}]_x = su, \\ &\rho_t + u_{tx} = 0, \end{aligned} \tag{C.27}$$

as a special case of (C.20)–(C.22). The dispersion relation (C.24) is greatly simplified by the fact that $c(k^2)$ turns out to vanish under our specialized assumptions, so that we can solve immediately to get

$$\sigma(k^2) = \frac{-b(k^2)}{2\mu k^2}, \tag{C.28}$$

where

$$b(k^2) = \gamma\tau_1 k^4 + (1 - \tau_1 - \tau_2)k^2 + s. \tag{C.29}$$

Clearly, here, for the spatially homogeneous case, $b(0) = s > 0$ so that $\sigma(0) < 0$; that is, the uniform solution is stable. For pattern formation, the system should exhibit unstable modes with $\sigma(k^2) > 0$ for some range of $k^2 \neq 0$ (clearly here σ is real). This requires at least that $\tau_1 + \tau_2 > 1$, and that the minimum value of b is negative:

$$b_{\min} = s - \frac{(\tau_1 + \tau_2 - 1)^2}{4\gamma\tau_1} < 0. \tag{C.30}$$

This condition, rewritten in terms of the original dimensionless parameters τ, λ, γ and s, becomes

$$\tau^2 - \tau(1+\lambda)^2[1 + \gamma s(1+\lambda)] + \frac{(1+\lambda)^4}{4} > 0, \tag{C.31}$$

which implies that spatial patterns will evolve only if

$$\tau > \tau_c = (1+\lambda)^2 \frac{1 + \gamma s(1+\lambda) + \{[1+\gamma s(1+\lambda)]^2 - 1\}^{1/2}}{2}. \tag{C.32}$$

This dispersion relation holds no great surprises for us; it has essentially the same, parabolic, form as those we encountered in connection with reaction-diffusion equations. The cell traction τ

is a *natural bifurcation parameter* in view of its central role in morphogenesis (see [147, 148, 289]), so that the surface $\tau = \tau_c(\lambda, \gamma, s)$ is the bifurcation surface between spatial homogeneity and heterogeneity. As soon as τ increases beyond the critical value τ_c, the value which first makes $b(k^2)$ zero, the uniform steady state bifurcates to a spatially unstable state; and for τ somewhat above the critical value, there is a range of unstable wavenumbers k, namely all those for which $\sigma(k^2) > 0$. These unstable wavenumbers are found from

$$k_1^2 < k^2 < k_2^2 \tag{C.33}$$

where

$$k_1^2, k_2^2 = \frac{(\tau_1 + \tau_2 - 1) \pm \{(\tau_1 + \tau_2 - 1)^2 - 4s\gamma\tau_1\}^{1/2}}{2\gamma\tau_1} \tag{C.34}$$

and

$$\tau_1 = \frac{\tau}{1+\lambda}, \quad \tau_2 = \frac{\tau(1-\lambda)}{(1+\lambda)^2}, \tag{C.35}$$

where τ, λ, γ and s must satisfy (C.32). In analogy to the reaction-diffusion situation, there is a fastest-growing linear mode which approximately predicts, in the one-dimensional model with random initial conditions, the ultimate nonlinear spatial pattern. Other ways of initiating the instability result in different preferred modes, as discussed in appendix A.4.

Further Analytical Approaches

The dispersion relation and patterns obtained in the above simplified model correspond to those found in reaction-diffusion equations; but as already indicated, other versions of the model, considering the effects of different combinations of parameters, result in different dispersion relations and thus potentially different spatial structures and pattern-forming possibilities. As the cell-matrix interaction equation is a *tensor* equation, the solutions of the mechanical system may be expected to have a wider class of singularities than vector systems such as reaction-diffusion equations, and hence more diverse spatial patterns. Few of the solution possibilities have yet been analysed in any depth.

A numerical study of the full model in one dimension has been performed by Perelson *et al.* [306], using a general-purpose partial differential equation solver, which confirmed the possibility of spatially heterogeneous and spatio-temporally oscillating patterns. They concentrated in particular on *mode selection* in models with many parameters, and proposed a technique, based on nonlinear least squares fitting to a dispersion relation, for determining parameter sets to isolate and 'grow' a pattern of a specific wavelength.

A nonlinear analysis of a simplified version of the model was performed by Maini and Murray [216]. In one dimension, an amplitude equation for the inhomogeneous steady state was derived using small parameter expansions, multi-time scales and the method of balancing harmonics. In two spatial dimensions, an even simpler caricature version, assuming small strains and hence replacing the force equation (C.21) with a nonlinear scalar equation in the dilation θ, was considered; and the possibility of stable solutions with particular symmetries which tesselate the plane, such as roll, rhombic and hexagonal solutions, was demonstrated. A further nonlinear multi-time scale perturbation procedure to study the evolution of spatio-temporal patterns was applied by Maini [214] to a more complicated one-dimensional version of the model equations

than in [216]. Maini also analysed a singular dispersion relation arising in the actual model equations, and showed that a small amplitude spatially heterogeneous steady state could evolve. These preliminary analyses all thus confirm the undoubted considerable self-organizing, pattern-forming potential of the mechanical model.

Coupling of Reaction-Diffusion and Mechanical Models An extension to the purely mechanical model has been proposed by Nagorcka *et al.* [265], who suggest the coupling of a mechanical system in the dermis to a reaction-diffusion system operating in the epidermis, to obtain more complex spatial patterns, as discussed in section 5.1.1. Numerical simulations of the model [265, 320] and linear analyses suggest that it is possible to isolate two spatial modes that superimpose giving rise to a pattern with *two* characteristic wavelengths; a nonlinear analysis [320] predicts solution behaviour in good agreement with the numerical solutions. A one-dimensional caricature of the model has also been studied [215] for some analytically more complex cases where there is a special relation between the two unstable wave numbers, giving rise to extra secular terms; and the possibility of either mode isolation or superposition of modes, depending on initial conditions and the model parameter values, was demonstrated.

Outlook Beyond the above-mentioned studies, not much analytical work on the mechanical and mechano-chemical models for morphogenesis has been performed, and the model equations still pose a considerable mathematical challenge, being much less well-understood than reaction-diffusion equations. The applicability of this general model, and variations thereof, must however continually be questioned and evaluated in the light of relevance to experiments. Furthermore, there is a wide range of other models, displaying self-organizing solution properties, that have been proposed for other morphogenetic situations, many of which are also analytically formidable and would merit an intensive analysis; several of these are indicated in the main text, and two are derived below. In the absence of a considerable and concerted research effort, the full mathematical implications of the solution behaviour of many of these systems will remain largely unexplored; and maybe this is just as well, for it is first and foremost biological understanding and correspondence with experiments that is required and sought, not 'fancy' mathematics. Only once a model has been shown to give a realistic (albeit inevitably simplified) account of the *actual* biological processes involved in some morphogenetic situation, may the serious, labour-intensive mathematical study of the complicated nonlinear model equations strictly be justified. Until then, preliminary analyses or numerical studies are often sufficient to gain the insight necessary to assess the potential biological applicability of the model.

C.2 Mechanochemical Model for Chondrogenic Condensations

The basic mechanical model for mesenchymal morphogenesis, described above, has been invoked to account for the generation of the sequence of cartilage condensations in the developing vertebrate limb [251, 289], as described in section 5.1.1. In this model, as we have seen, the motion of the cells, for instance through diffusion, and the external, tethering forces on the matrix play significant roles. A modification of the basic model has been proposed by Oster, Murray and Maini [290], incorporating chemical aspects and the added forces caused by osmotic pressure in the developing limb, but no cell motions other than convective transport by matrix

contraction. This model may, under certain simplified circumstances, reduce to a form mathematically analogous to a reaction-diffusion system (although the interpretations of the various terms are quite different) thus indicating that in terms of their potential for self-organization, the mechanochemical models are generalizations of reaction-diffusion-type systems. The demonstration of this mathematical equivalence is of interest to us, and motivates the brief derivation and presentation of the model equations below.

Formulation of Model Equations

Description of Condensation Mechanism In the mechanical models (see appendix C.1), the extracellular matrix (ECM) plays a passive role, serving only as an elastic substrate which can deform under cell-induced tractions and transmit stresses from one point to another. The alternative model to be considered here presents the ECM as an *active* participant in morphogenesis, capable of osmotic swelling and deswelling and hence of bringing cells into contact. Hyaluronic acid (HA) is a principal component of the ECM, and can exist as a polyelectrolyte gel in a swollen osmotic state. The condensation of chondrocytes (precursors of bones) begins with the secretion, by the cells, of an enzyme, hyaluronidase (HAase), which degrades HA, causing the gel to deswell; such enzyme secretion could result, for example, from the emergence of cells from the progress zone. This could lead to the osmotic collapse of the matrix, bringing the cells into close enough contact with each other to exert strong intercellular traction forces and initiate active contractions, which can generate organizing centres about which the cell aggregations characteristic of the early stages of chondrogenesis form (see the original paper [290], and also [16, 218]).

Mathematical Formulation The mathematical formulation of the model consists of five equations [290]: two describe the changes in cell and matrix density due to convective motion caused by cell traction; two equations account for the production, degradation and transport of the chemicals HA and HAase; and a force balance equation treats the different forces acting in the cell-matrix milieu, specifically, cell traction, osmotic swelling pressure, and the visco-elasticity of the matrix. Thus we assume firstly that mitosis and matrix secretion are not important during the condensation stage, and that the cells and the non-osmotic component of the ECM move only by convection, that is, they are passively dragged by the deformations of the matrix. Hence we get, for the cell density n and the matrix density ρ, with deformation $\mathbf{u}$:

Cell conservation equation:

$$\frac{\partial n}{\partial t} = -\nabla \cdot (n\mathbf{u}_t) \tag{C.36}$$

Matrix conservation — the non-osmotic component of the ECM:

$$\frac{\partial \rho}{\partial t} = -\nabla \cdot (\rho\mathbf{u}_t) \tag{C.37}$$

The conservation equation for HA, with concentration h, incorporates not only convection but also S_h, the secretion rate of HA by the cells, and D_h, the rate of degradation of HA by HAase. This leads to a model equation of the form:

The hyaluronate component of the ECM (HA):

$$\frac{\partial h}{\partial t} = -\nabla \cdot (h\mathbf{u}_t) + S_h - D_h, \tag{C.38}$$

where explicit analytical forms for the functions S_h and D_h are chosen based on the expected behaviour of these terms, for example

$$S_h - D_h \equiv F_1(n,h,a) = \frac{B_0 hn}{(K_0 + h^2)(K_1 + n)} - \frac{B_1 ha}{K_2 + h}, \tag{C.39}$$

where B_0, B_1, K_0, K_1 and K_2 are constants.

For the enzyme HAase, with concentration a, we consider diffusion (diffusion coefficient D), production by the cells, at rate S_a, and degradation, at a rate D_a; this yields a conservation equation given by:

Hyaluronidase (HAase):

$$\frac{\partial a}{\partial t} = D\nabla^2 a - \nabla \cdot (a\mathbf{u}_t) + S_a - D_a, \tag{C.40}$$

where suitable parametrizations of the functions are given by

$$S_a - D_a \equiv F_2(n,h,a) = \frac{C_0 h^2 n}{K_3 + h^2} - C_1 a, \tag{C.41}$$

with, as usual, C_0, C_1 and K_3 constant.

Lastly, the force balance equation, which incorporates the viscous drag forces between the solid and fluid components of the tissue, the passive elastic forces of the cells and the matrix, the osmotic swelling pressure and the active cell traction forces, is written in the form:

Force balance (stress) equation:

$$\begin{aligned} \nabla \cdot \sigma \;=\; \nabla \cdot \{ & \underbrace{E(\varepsilon - L_1 \nabla^2 \varepsilon)}_{\text{elastic strain}} + \underbrace{\mu \varepsilon_t}_{\text{viscous pressure}} \\ & - \underbrace{\Pi(h,\varepsilon)\mathbf{I}}_{\text{osmotic pressure}} + \underbrace{\tau(n)(n + L_2 \nabla^2 n)\mathbf{I}}_{\text{cell tractions}} \} = 0, \end{aligned} \tag{C.42}$$

where $\varepsilon = \nabla \mathbf{u} + \nabla \mathbf{u}^T$ is the (linear) strain, E the Young's modulus, $\mathbf{I}$ the unit tensor, μ the viscosity, and the coefficients L_1 and L_2 govern the magnitude of the second-order strains arising from long-range interactions. For computational purposes, particular forms of the tractional and osmotic functions $\tau(\cdot)$ and $\Pi(\cdot,\cdot)$, corresponding to the expected qualitative behaviour, are chosen; for example

$$\tau(n) = \frac{\tau}{K + n^2}, \quad \Pi(h,\varepsilon) = \frac{\Pi h^2}{1 + \varepsilon} \tag{C.43}$$

(in one dimension, for scalar strains), where τ, K and Π are constant parameters. For a fuller and more biologically motivated derivation of the model equations, see the original paper [290].

A Simplified Version of the Model

A preliminary indication of the behaviour of the system is obtained by assuming that only small strains are generated; this allows us to replace the equations (C.36) and (C.37) by their integrated, linearized forms (in one spatial dimension), namely

$$n \approx N(1-\varepsilon), \tag{C.44}$$

$$\rho \approx M(1-\varepsilon), \tag{C.45}$$

where N and M are positive constants. A further simplification is obtained by relating the HA-generated osmotic term in (C.42) to the concentration of HAase, as the presence of HA stimulates the cells to produce the enzyme. Thus we can make the replacement

$$\frac{\Pi h^2}{1+\varepsilon} = \frac{P_0}{1+\varepsilon} - \alpha a, \tag{C.46}$$

where P_0 and α are constants. The inverse relationship between strain and osmotic pressure is hereby contained in the term $P_0/(1+\varepsilon)$, while the rationale for the other term in the substitution is that the presence of h stimulates the production of a, which in turn degrades h; this may be modelled simply by a decay term of the form $-\alpha a$.

Introducing these approximations into (C.36)–(C.40) and the integrated form of (C.42) (in one spatial dimension) yields an uncoupled equation for the stress ε:

$$\begin{aligned} \mu\varepsilon_t &= EL_1\varepsilon_{xx} + [\sigma_0 - E\varepsilon - \frac{\tau N(1-\varepsilon)}{K+[N(1-\varepsilon)]^2} + \frac{P_0}{1+\varepsilon} - \alpha a] \\ &= EL_1\varepsilon_{xx} + F(\varepsilon, a), \end{aligned} \tag{C.47}$$

where σ_0 is a constant of the spatial integration of (C.42), corresponding to a constant stress at the boundary of the limb.

The equation for the HAase may also be simplified: the production term in (C.40), which was given in (C.41) as $C_0h^2n/(K_3+h^2)$, increases with strain ε, as higher strains accompany dense cell packing and the production of HAase is clearly higher when the cells are densely packed. This can be incorporated into a simple linear growth term with production rate constant ν, to yield a modified equation for the HAase, as

$$a_t = Da_{xx} + \nu\varepsilon - \omega a, \tag{C.48}$$

where $\omega = C_1$ from (C.41).

Comparison with Reaction-Diffusion Equations Thus our model system (C.36), (C.37), (C.38), (C.40) and (C.42), has been reduced to a greatly simplified, caricature pair of equations for the strain ε and the HAase concentration a, which to some extent do, however, capture the essential physics of the mechanochemical process. Of interest in this system is the *form* of the equations, which are formally identical to a reaction-diffusion system. The solution properties are thus analogous to those of reaction-diffusion equations, so that this model displays all the symmetry-breaking, self-organizing characteristics which we have learnt to associate with those equations. As this result is obtained through approximation and simplification, we may expect

that the full pattern-forming potential of the mechanochemical model is much wider than that of reaction-diffusion systems, which are contained as a special case.

The analogy is, of course, purely formal; the 'reaction' term $F(\varepsilon, a)$ in (C.47) has no obvious counterpart in chemical kinetics, but contains the strain production terms associated with the osmotic, elastic and cell traction forces. Nevertheless, the mathematical equivalences are of particular interest, as they indicate that, in spite of fundamentally different physical motivation and postulates, different self-organizing mechanisms may ultimately be equivalent as far as their basic symmetry-breaking, pattern-forming properties are concerned. Of course, the mechanical models can only be reduced to reaction-diffusion form under very special assumptions; there are important cases, such as those discussed in appendix C.1, for which the differential equations are fundamentally different from the reaction-diffusion type.

Lateral Inhibition Interpretation of Mechanochemical Models

The underlying equivalence of the self-organizing models may readily be observed in the context of a lateral inhibition interpretation [287, 288]. We have already noted (see section 3.2.2) the physical interpretation of reaction-diffusion instabilities and patterning in terms of local autocatalysis and long-range inhibition, as emphasized by the activator-inhibitor models of Gierer and Meinhardt [114]. A similar interpretation for the mechanochemical models is perhaps slightly less obvious, but also inherently plausible.

For the general mechanical model discussed in the previous section [289], for example, *local autocatalysis* is accomplished in two ways:

- As contractile cells aggregate, the strength of the contractile region increases.
- As the actomyosin fibres which generate the cells' contractile forces shorten, they become stronger, in consequence of the 'sliding filament' mechanism underlying actomyosin contraction; this leads to enhanced contraction and an inherently unstable mechanism.

This effect is counteracted by the lateral inhibition contained in the model, arising in several ways:

- The negative slope of the actomyosin stress-strain curve means that cells which have been dilated by the contraction of their neighbours are mechanically weaker, amounting to a lateral inhibition of their contractile ability.
- The elasticity of the substratum to which cells are attached causes an attenuation of the range of a contractile focus.
- Long-range elastic interactions of the cells themselves, transmitted through their filopodia or fibrous materials such as the ECM, give rise to biharmonic ∇^4 terms in the model, which act to attenuate the stress and produce lateral inhibition.
- Recruitment of cells by convection into an aggregation centre depletes the neighbouring regions, thus creating a surrounding mechanically weaker zone around a contractile focus.

Thus, as for chemically reacting and diffusing systems, the mechanical systems are characterized by activation on a short scale, accompanied by the creation of a surrounding inhibited region. There is ample evidence throughout this thesis that the capability for self-organization tends to be associated with such qualitative behaviour, thus providing a heuristic basis for the underlying equivalence of different self-organizing models.

A consequence is that this equivalence between self-organizing models implies that different models tend to predict similar patterns, as mechanisms associated with lateral inhibition all establish a 'zone of influence' around each focus, which enforces a characteristic spacing. This frequently makes it difficult to distinguish between their pattern-forming predictions, which tend generally to be based on linearization and hence similar linear eigenvalue problems anyway. The physical foundations, for example of reaction-diffusion *versus* mechanical models, are quite different, however, so that different models suggest different *experimental* interventions to test their assumptions — for example, culturing limb rudiments in the presence of cytochalasin, which weakens intracellular cytoskeletal activity and hence putative tractional forces, would be expected to affect condensation and chondrogenesis if a mechanical mechanism was operative, but would not necessarily disrupt patterning in the case of reaction-diffusion-based mechanisms.

We have considered the mathematical basis for two versions of possibly the major mechano-chemical model for mesenchymal morphogenesis, embodying the cooperation of cells in their own patterning, and thus simultaneous pattern-formation and morphogenesis. The model was treated in some depth to demonstrate the type of mathematical formulation and analysis involved in models of cellular interactions in pattern formation and morphogenesis. The Oster-Murray model is prominent in theoretical studies of morphogenesis, but, as indicated in chapter 5, is by no means the only study to have incorporated mechanical effects of cell behaviour; in particular, it is restricted to mesenchymal cells, whereas epithelial cells forming cell sheets have considerably different morphogenetic properties.

C.3 A Model for Chemotaxis

The most general considerations of the morphogenetic properties of mesenchymal cells consider both the motion of the individual cells and their mechanical interactions with the extracellular matrix. Pattern-forming potential has, however, been demonstrated for more restricted models, as we have seen above, for example if we consider only haptotaxis (motion along an adhesive gradient) in connection with cell traction, and neglect diffusive and convective motions; or alternatively ignore haptotaxis, and allow cells to move only by convection. Another possibility for patterning is by purely individual cell motions in response to a chemical gradient, that is *chemotaxis*, neglecting any mechanical forces and the ECM. Chemotactic behaviour has been observed experimentally and modelled theoretically, with some success, for some bacteria and for the cellular slime mould, in particular by Keller and Segel [185, 186]; these models have since been extensively investigated analytically and developed further (see for example [58, 184, 261]). The Keller-Segel model for slime mould chemotaxis is briefly outlined in section 5.1.2. For a good pedagogical introduction to these models, see [87].

The application of chemotactic considerations to morphogenesis of multicellular organisms is, however, fairly recent, having been introduced by Oster and Murray in 1989 [288] — this is possibly surprising, as the self-organizing properties of chemotactic systems have long been

recognized. The original formulation of the model considered an application to chondrogenesis, the sequential generation of cartilage pattern in the developing limb [288]; but later applications have been proposed to pigmentation patterning on alligators [253] and snakes [256]. The model equations are always essentially the same, and are all based on the assumption that cell aggregation is guided by a taxis, with cells migrating towards higher concentrations of a chemoattractant that they themselves secrete.

Model Formulation and Outline of Analysis The model formulation is quite straightforward, especially in the light of our previous experience with similar models, and involves the conservation equations for the cell density $n(\mathbf{x},t)$ and chemoattractant concentration $c(\mathbf{x},t)$:

Cell conservation:

$$\frac{\partial n}{\partial t} = \underbrace{D_n\nabla^2 n}_{\text{random motility}} - \underbrace{\alpha\nabla\cdot(n\nabla c)}_{\text{chemotaxis}} + \underbrace{rn(N-n)}_{\text{mitotic proliferation}}, \tag{C.49}$$

where D_n is the cell motility coefficient, α the chemotactic parameter and r and N the linear mitotic rate and carrying capacity for cell proliferation, respectively. Clearly, the chemotactic flux is proportional both to the density of cells and the gradient of attractant.

Chemoattractant conservation:

$$\frac{\partial c}{\partial t} = \underbrace{D_c\nabla^2 c}_{\text{diffusion}} + \underbrace{\frac{Sn}{\beta+n}}_{\text{secretion by cells}} - \underbrace{\mu c}_{\text{linear decay}}, \tag{C.50}$$

where D_c is the diffusion coefficient of chemoattractant, and μ the decay rate of c. The chemoattractant production is assumed to saturate according to Michaelis-Menten kinetics, with parameters S corresponding to the maximum secretion rate of the chemicals by the cells and β the Michaelis constant associated with the chemoattractant production. As usual, all parameters are positive.

The analysis of this model proceeds in the same way as we have seen before. It is useful first to nondimensionalize the equations, before a linear stability analysis is performed, to obtain the conditions for pattern formation, the dispersion relation and the expected pattern wavelength [288]. Numerical solutions in one and two dimensions, with particular consideration of mode isolation, the effects of individual parameters and domain scale and geometry, have been obtained [217], and the effect of pattern formation in a growing domain has been considered, with particular application to the generation of the typical pattern elements on snakes [256]. An analytical study of propagating spatially heterogeneous patterns, obtaining estimates for the pattern wavelength and the speed of spread, has also been performed [260], including a comparison of the analytical results with numerical simulations of the full system. Thus a fair amount of effort at analytical and numerical understanding of the solution behaviour, coupled with an attempt at realistic biological applications of this proposed mechanism [253], has been made; but it need not be elaborated in too much detail here, as the techniques are essentially analogous to those we have already seen in the appendices.

Chemotaxis as a Lateral Inhibition Mechanism It may again be appropriate to indicate the connection of this model with the general paradigm of lateral inhibition [287]. The evolution

of pattern due to the chemotactic aggregative driving force is in fact intuitively quite clear [252]: A small positive perturbation in cell density from an initially uniform steady state distribution implies that more chemoattractant is produced locally because of the second term in equation (C.50). This induces a local gradient in c, so that cells tend to move up this gradient. The chemotaxis is thus the *autocatalytic* effect which tries to increase the local aggregation. The local activation is counteracted by the dissipative tendencies of diffusion, which tends to smooth out spatial variations and acts as an *inhibitory* effect; furthermore, aggregation of cells depletes the neighbouring regions and thus introduces additional lateral inhibition.

Once again we are able to interpret a self-organizing process in terms of lateral inhibition; and, in the light of our previous discussions, we expect the basic pattern-forming properties to be essentially analogous to those we have studied before. This analysis thus again serves to emphasize the universality of the concepts of self-organization and lateral inhibition, and their applicability to a wide variety of different pattern-forming and morphogenetic mechanisms.

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